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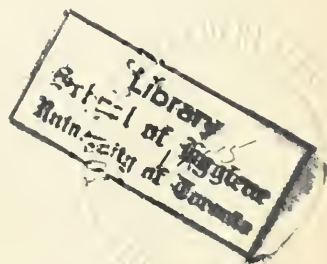
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STUDIES ON THE BLOOD PROTEINS, III

ALBUMIN-GLOBULIN RATIO IN ANTITOXIC IMMUNITY

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INTRODUCTION

In a previous communication,[1] we presented the results of some experimental observations bearing on the relationship between the globulin content of an animal's serum and the grade of immunity developed in it by immunization with various bacteria. These observations brought out several interesting points concerning the relation of the serum globulins to the antibodies, and especially emphasized the lack of parallelism between the globulin content of the blood and the concentration of antibodies. During the past year, we have directed our efforts to a consideration of this problem in its bearing on antitoxic immunity. This subject possesses more than academic interest because of the constant association of the antisubstance with the serum globulin, and because of the practical application that has been made of this knowledge in the preparation and concentration of antitoxic serums.

A restudy of this problem by improved methods was thought desirable because only a few investigations have been made of the globulin variations in relation to potency, and these, for reasons which will be given, are somewhat incomplete, and, therefore, leave certain of the conclusions derived open to doubt.

Brodie[2] was among the first to show that diphtheria antitoxin was completely precipitated from a solution by any means which removed the globulins, and his observations were extended by Belfanti and Carbone[3] and by Seng.[4] But the first notable advance in this study was made by Hiss and Atkinson[5] who estimated the globulin content of the serum of a large number of horses at different stages of immunization against diphtheria toxin. As a result of

[1] Hurwitz, S. H., and Meyer, K. F.: Jour. Exper. Med., 1916, 24, p. 515.
[2] Jour. Path. and Bacteriol., 1896, 4, p. 460.
[3] Abstr., Centralbl. f. Bakteriol., 1, 1898, 23, p. 906.
[4] Ztschr. f. Hyg. u. Infektionskrankh., 1899, 31, p. 513.
[5] Jour. Exper. Med., 1900, 5, p. 47.

these experiments, these workers arrived at the conservative conclusion that a low potency coincided with a low globulin content, but that it was not possible to regard the absolute amount of globulin as an index of the antitoxin content of the serum. Similar studies were made by Ledingham[6] and by Gibson and Banzhaf.[7] The former concludes from the data furnished by the immunization of a horse and a goat with diphtheria toxin that there would seem to be "some intimate relation between the amount of antitoxin developed and the quantity of the globulins." Gibson and Banzhaf are still more guarded in their conclusions. These workers state that while the greatest rise in the serum globulin was usually coincident with maximum antitoxic potency, the extent of this increase was practically independent of the antitoxin potency when the results on more than 1 horse were contrasted. They, therefore, are inclined to the view that there may be no relation between the absolute or percentage increase of the serum globulin and the antitoxic potency in the plasma of different horses; indeed, they have shown that the increase in the serum globulin of refractory horses may surpass that in the plasma of some of those yielding a high antitoxin. Similar deductions were made by Banzhaf and Famulener[8] in the immunization of goats. They found that the total protein content and the protein partition may be normal at a time when the animal shows the maximum number of antitoxic units.

From our own experiments, we can subscribe to the view that the globulin change is not a necessary concomitant of the elaboration of antibodies, although it is now well established that this protein fraction may increase strikingly during the process of immunization. Our conclusions have been derived from parallel studies of the serum protein fractions and of the antitoxin content of a number of different animals immunized not only with diphtheria toxin, but also with the soluble toxins of the bacilli of tetanus and of botulism. In general, the results obtained in the different animals and with the different soluble toxins point in the same direction and lend support to our previous findings in animals immunized with bacteria and their toxins, namely, that the curves of serum globulin increase and the development of antitoxin potency do not run parallel. Our observations, furthermore, furnish additional evidence that there is some relation between the shock and intoxication caused by the injection of the toxin and the rise in globulins, although we have found an interesting deviation from this reaction in the case of the toxin of the bacillus of botulism. The details of these latter experiments will be given in their proper place, but it may be well to state here that with this particular toxin the immediate result of the inoculation is an increase not of the globulin, but of the albumin fraction. In those instances, however, in

[6] Jour. Hyg., 1907, 7, p. 65.
[7] Jour. Exper. Med., 1910, 12, p. 411.
[8] Collect. Stud. Research. Lab., Dept. Health, N. Y., 1915, 8, p. 208.

which a rise in globulins was found to occur, the increase usually took place at the expense of the albumin fraction, so that the effects of bacterial and soluble toxins are also not unlike in this respect.

Methods

The experiments were carried out on the horse, dog, goat, and rabbit. The use of these different types of animals is desirable in such a study because, as von Behring has shown, the various animals differ markedly in susceptibility to toxin injections and, therefore, in their degree of response, the dog being the most susceptible and the horse and goat the least susceptible animals. Rabbits do not develop much immunity, but their use served to control our studies on bacterial infection and immunity in which the rabbit was used to the exclusion of all other animals. With the exception of the horse, the experimental animals were kept under constant conditions of diet and activity. The goats were permitted to graze all day, specimens of blood for the protein determinations being collected before the animals were turned out in the pasture. The horses were kept and immunized by the Cutter Laboratory, Berkeley, Calif., and it is a pleasure to acknowledge our indebtedness to Dr. Harry Foster of this laboratory for supplying us regularly with the specimens of blood tested and for many other favors in connection with this work.

For some of the toxins used—diphtheria, tetanus, and botulism—we are indebted to Dr. Foster, to Dr. Krumwiede and Dr. Banzhaf of the Health Dep't of New York City, and to Dr. Dickson of Leland Stanford University.

All of the animals, regardless of type, were immunized with toxin of a known M. L. D. content. The goats were immunized with small but gradually increasing doses of toxin, the amounts chosen varying slightly from those given by Ledingham.[6] A similar procedure applied to dogs soon had to be abandoned because the animals became severely intoxicated and died. Attempts to immunize them with balanced mixtures were successful only when the animals were permitted to recuperate completely from the local and general reactions. In the 2 successful experiments, about 1 month had to elapse between the inoculation of 0.5 c.c. toxin and the 2nd injection of the same amount. The production of a basic immunity in the dog is, therefore, difficult, but, when once attained, amounts of toxin equal to double the initial dosage may be administered subcutaneously in 3-day intervals and no harm be done. We cannot, therefore, agree with Wernicke[9] that dogs are readily immunized, at least when highly potent diphtheria toxin is employed, such as is used at present for the production of antitoxin in the horse.

The rabbits were immunized subcutaneously with toxins which had been attenuated with Lugol's solution, according to the methods recommended by Roux and now used at the Pasteur Institute. In the employment of tetanus, our experiments were successful only after we had diluted the toxin in the proportions suggested by Roux, and permitted the detoxification to continue for at least from one half to three quarters of an hour. Shorter periods of detoxification produced a toxin mixture which caused symptoms of tetanus after a fairly long incubation period.

The antitoxin content of the serums of the experimental animals was determined by several methods. At first, the method commonly employed in North America was used,[10] and a few tests with the Römer intracutaneous

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[9] Ztschr. f. Hyg. u. Infektionskrankh., 1892, 28, p. 43.
[10] Bull. 21, Hyg. Lab., U. S. P. H. and M.-H. S., 1902.

method[11] were made. However, Zingher's modification[12] of the intracutaneous test was used for the greater part of the determinations. This method has proved to be reliable and highly satisfactory for experimental work, more particularly for the determination of an antitoxin content below 1 unit. The results of this procedure were further checked by the United States Standard Method, for this could be done economically and in a relatively short time, since we were fortunate enough to obtain well tested L⁺ toxins at regular intervals from the Cutter Laboratory.

The potency of the antitetanic serums was roughly estimated by the American method.[13] Properly standardized L⁺ toxin was obtained from the Cutter Laboratory. For the determination of the antitoxin content of the dogs immunized with botulism toxin, we chose arbitrarily an amount of toxin (1:800-1:1500) which killed a guinea-pig, weighing 250-350 gm., in 48 hours. Analogous to the standardization of tetanus antitoxin, that amount of antiserum was considered to possess one tenth of an immunity unit which could save the life of a guinea-pig, weighing 250-350 gm., in 48 hours. This, to be sure, is a very rough method, but the only one possible, because of the unavailability of a standard serum.

All the determinations of the albumin, globulin, and nonprotein fractions were made by the microrefractometric method of Robertson.[14]

I. IMMUNIZATION WITH DIPHTHERIA TOXIN

Because of the practical bearing of the relation of the immune bodies to the globulins in the concentration of diphtheria antitoxin from the horse, our attention was first directed to some experimental observations on this animal. From the analytic studies of a number of workers,[15] it has been determined that in the horse there is normally present a higher percentage of globulin in proportion to albumin. According to these observations and our own, the globulins constitute about 50% of the total proteins, giving the protein quotient a value of approximately 1 (Table 1). These studies were then extended to include the goat, dog, and rabbit. In the case of the latter 2 animals, it has been shown in previous communications that the normal protein quotient is

TABLE 1

SERUM PROTEINS OF NORMAL HORSES

Date	Horse	Total Protein, %	Total Albumin, %	Total Globulin, %	Albumin of Total Protein, %	Globulin of Total Protein, %	Non-protein Constituents, %	Protein Quotient
Jan. 6	1	5.8	3.3	2.5	57	43	1.5	1.3
Jan. 6	2	6.6	3.9	2.7	59	41	1.4	1.4
Jan. 13	115	7.4	3.7	3.7	50	50	1.2	1
Average......		6.6	3.6	2.9	55	44	1.4	1.2
R. M. Jewett*		7.5	4	3.5	53	47	1.65	1.1

* Jour. Biol. Chem., 1916, 25, p. 21.

[11] Ztschr. f. Immuitätsforsch., 1909, 3, p. 49.
[12] Jour. Infect. Dis., 1916, 19, p. 557.
[13] Bull. 43, Hyg. Lab., U. S. P. H. and M. H. S., 1908.
[14] Jour. Biol. Chem., 1915, 22, p. 223.
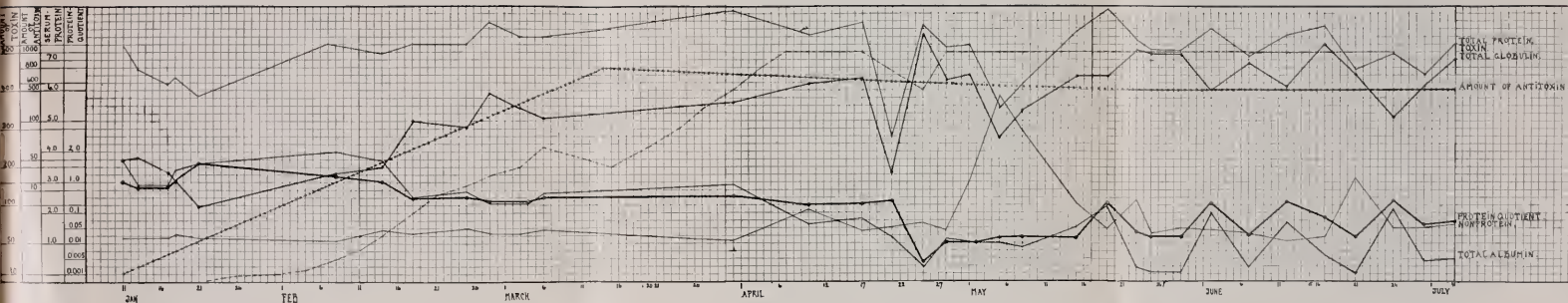[15] Jewett, R. M.: Jour. Biol. Chem., 1916, 25, p. 21.

Chart 1.—Horse injected with diphtheria toxin.

method" were made. However, Zingher's modification[12] of the intracutaneous test was used for the greater part of the determinations. This method has proved to be reliable and highly satisfactory for experimental work, more particularly for the determination of an antitoxin content below 1 unit. The results of this procedure were further checked by the United States Standard Method, for this could be done economically and in a relatively short time, since we were fortunate enough to obtain well tested I* t

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* Jour. Biol. Chem., 1916, 25, p. 21.

[11] Ztschr. f. Immuitätsforsch., 1909, 3, p. 49.
[12] Jour. Infect. Dis., 1916, 19, p. 557.
[13] Bull. 43, Hyg. Lab., U. S. P. H. and M. H. S., 1908.
[14] Jour. Biol. Chem., 1915, 22, p. 223.
[15] Jewett, R. M.: Jour. Biol. Chem., 1916, 25, p. 21.

much higher than in the horse (see Table 2) ; whereas the protein quotient of the goat has been found to be closer to that of the horse. It is well to bear in mind, however, that fluctuations may occur even in normal animals, and it is well, therefore, in all instances to make some preliminary observations on the normal animal before proceeding to the inoculation experiments.

TABLE 2

PERCENTAGES OF SERUM PROTEINS IN NORMAL RABBITS

Rabbit	Total Protein, %	Total Albumin, %	Total Globulin, %	Albumin of Total Protein, %	Globulin of Total Protein, %	Nonprotein Constituents, %	Protein Quotient
327	5.3	4	1.3	75	25	2.1	3
436	6.1	5.2	0.9	85	15	1.3	5.6
449	7.6	5.3	2.3	69	31	1.1	2.2
458	6.3	5.5	0.8	87	13	1.2	6.7
541	6.7	4.8	1.9	71	29	1.2	2.4
570	7	5.2	1.8	74	26	1.3	2.8
571	5.8	3.7	2.1	64	36	1.2	1.7
575	6	4.8	1.2	80	20	1.3	4
576	7	5	2	71	29	1.1	2.4
577	6.6	5.3	1.3	80	20	1.2	4
579	7.3	5	2.3	68	32	1.1	2
580	6.6	5.5	1.1	83	17	1.2	4.9
588	5.8	5	0.8	86	14	1.3	6.1
604	5.3	4.2	1.1	80	20	1.4	4
619	6.7	4	2.7	60	40	1.5	1.5
626	5	3.7	1.3	74	26	1.6	2.8
680	5.8	3.2	2.6	60	40	1.7	1.5
681	5.1	3.1	2	60	40	1.6	1.5
700	5.9	3.9	2	66	44	1	1.5
712	6.1	3	3.1	50	50	1.4	1
713	6.1	3.3	2.8	54	46	1.2	1.2
714	5	2.4	2.6	50	50	1.9	1
715	5.7	3.9	1.8	68	32	1.7	2.1
720	6.1	4	2.1	70	30	1.4	2.3
Average	6.1	4.2	1.8	70	30	1.3	2.8

A. *Immunization of the Horse.* One complete experiment was carried out over a period of 7 months. The detailed observations of this complete study are given in Table 3 and Chart 1. A few points, however, may deserve additional emphasis. At the beginning of the experiment (January 11), the protein quotient was found to be 1, and rose to its highest point, 1.6, 10 days later. The 1st depression in the quotient (rise in globulins) occurred about 4 weeks later, following 8 injections (125 c.c.) of a toxin with a toxicity of 200 M. L. D. Following the repeated injections of large amounts of toxin, a considerable grade of immunity was obtained. A determination of the antitoxic value, on March 14, showed the presence of 800 units. At this period, the protein quotient was about 0.5, and apparently continued unchanged, notwithstanding the repeated injection of larger doses of toxin. The globulins continued to rise, reaching their highest value on April 25, at a time when the animal's hyperimmunity was beginning to diminish, as was evidenced by a reduction in the number of antitoxic units. At this period, it will be noted, from Table 3 and Chart 1, that the globulins constituted 99.6% of the total proteins. Despite the fact that the number of antitoxin units diminished still further toward the end of the experiment, the globulin percentage nevertheless remained almost at a maximum point until the end of our observations. Some fluctuations were encountered, as will be clearly seen in the chart, but these do not appear to have any definite relationship to the periods of inoculation.

TABLE 3

Effect of the Injection of Diphtheria Toxin on the Blood Proteins in the Horse

Date	Amount of Toxin, C.c.	M. I. D.	Amount of Anti- toxin, Units	Tem- pera- ture	Total Protein, %	Total Albumin, %	Total Globulin, %
Jan. 11	...	...	...	....	7.4	3.7	3.7
Jan. 13	...	...	...	....	6.4	2.9	3.8
Jan. 17	...	...	...	....	6.2	2.9	3.3
Jan. 18	...	...	...	....	6.4	3.4	3
Jan. 21	...	200	...	....	5.8	3.6	2.2
Jan. 22	2						
Jan. 24	5						
Jan. 26	8	...	...	38.9			
Jan. 31	10						
Feb. 4	15	...	...	38.8			
Feb. 7	25	...	...	38.0			
Feb. 8	...	...	...	38.3	7.3	4	3.3
Feb. 11	40	...	...	37.9	...	...	...
Feb. 14	60	...	...	38.6	7.2	3.7	3.5
Feb. 18	90	...	...	39	7.5	2.5	5
Feb. 21	125	...	...	39.4			
Feb. 25	150	...	...	38.8	7.5	2.7	4.8
Feb. 28	175	...	. .	38.9	8.2	2.3	5.9
Mar. 3	200	...	...	38.6	7.7	2.3	5.4
Mar. 6	250	...	...	39.5	7.7	2.6	5.1
Mar. 10	...	...	...	38.0	...	...	...
Mar. 14	...	...	800	38.6	...	...	...
Mar. 15	200	300	...	39			
Mar. 17	...	...	...	....	...	...	...
Mar. 20	250	...	...	38.8			
Mar. 24	300	...	...	38.6	...	...	...
Mar. 27	350	...	...	38.4			
Mar. 31	400	200	...	39.3	8.5	2.9	5.6
Apr. 3	450	200	...	39.0			
Apr. 7	500	...	...	38.9	...	...	...
Apr. 10	500	200	...	38.2			
Apr. 14	500	200	...	38.1			
Apr. 17	500	...	...	....	8.2	1.8	6.4
Apr. 21	...	...	...	37.9	4.5	1.2	3.3
Apr. 24	...	...	600	37.9	...	...	...
Apr. 25	400	200	...	39.2	8.1	0.3	7.8
Apr. 28	500	200	...	....	7.4	1.1	6.3
May 1	500	200	...	38.9	7.5	1	6.5
May 5	500	200	...	38.5	5.4	1	4.4
May 8	500	200	...	38.2	6.2	0.9	5.3
May 12	500	200	...	38.6			
May 15	500	200	...	38.6	7.9	1.5	6.4
May 19	...	...	...	38.2	8.6	2.2	6.4
May 23	...	...	500	38.1	7.6	0.2	7.4
May 24	500	...	...	38.4			
May 25	...	...	...	....	7.3	0.1	7.2
May 29	500	...	...	38.8	7.3	0.1	7.2
June 2	...	...	...	38.1	8	2	6
June 7	500	...	500	38.7	7.1	0.2	6.9
June 12	500	200	...	38.3	7.8	1.7	6.1
June 17	...	...	...	37.5	8.1	0.6	7.5
June 20	...	...	500	38.3	...	...	...
June 21	500	...	...	38.0	6.6	0.1	6.5
June 26	500	...	...	38.7	7.2	2.1	5.1
June 30	...	...	...	37.8	6.5	0.4	6.1
July 4	...	...	500	38.2	7.5	0.5	7

TABLE 3—Continued

EFFECT OF THE INJECTION OF DIPHTHERIA TOXIN ON THE BLOOD PROTEINS IN THE HORSE

Albumin of Total Protein, %	Globulin of Total Protein, %	Non-protein Constit-uents, %	Protein Quotient	Remarks
50	50	1.2	1	Bled 6 liters of blood
43	57	1.2	0.77	On Jan. 14, injection of 5,000,000 'antisuppurin'
46	54	1.2	0.8	
53	47	1.3	1.1	On Jan. 21, injection of 15,000 units of diph-
62	38	1.2	1.6	theria antitoxin and 15,000 units of tetanus antitoxin
56	44	1.1	1.2	
..	..	...	....	100 million antisuppurin
51	49	1.4	1	Bled before injection of toxin
33	67	1.3	0.5	100 million antisuppurin
36	64	1.5	0.56	1500 units of tetanus antitoxin injected
30	70	1.3	0.43	.
30	70	1.3	0.42	100 million antisuppurin
33	67	1.4	0.5	
..	..	...	.....	100 million antisuppurin
..	..	...	....	Bled 9 liters for plasma
..	..	...	....	100 million antisuppurin
..	..	...	....	1500 units tetanus antitoxin
34	66	1.1	0.51	
..	..	...	....	100 million antisuppurin
21	79	1.4	0.26	
26	74	1.1	0.36	1500 units tetanus antitoxin
..	..	...	....	Bled 9 liters for plasma
0.4	99.6	1.6	0.004	
1.5	98.5	1.4	0.015	
1.3	98.7	3	0.015	
1.8	98.2	5.8	0.02	1000 units tetanus antitoxin
1.7	98.3	4.7	0.02	
1.9	98.1	2.3	0.02	
25	75	1.5	0.3	500 units tetanus antitoxin
3	97	2.4	0.03	Bled 9 liters
2	98	1.3	0.02	
2	98	1.5	0.02	
25	75	1.4	0.33	1500 units tetanus antitoxin
3	97	1.3	0.03	Bled 9 liters
22	78	1.1	0.28	
8	92	1.2	0.08	1000 units tetanus antitoxin
..	..	...	....	Bled 9 liters
2	98	3.1	0.02	
30	70	1.5	0.4	
6	94	1.5	0.06	1000 units tetanus antitoxin
7	93	1.6	0.07	Bled 9 liters .

TABLE 4

Serum Proteins of Antitoxic Horse Serum

Date	Name of Horse	Amount of Antitoxin, Units	Total Protein, %	Total Albumin, %	Total Globulin, %
		Dec. 7 400			
Dec. 20	Dolly		5.2	2.6	2.6
		Dec. 21 400			
Dec. 23	Dolly		5.1	2.4	2.7
Dec. 27	Dolly		5	1.5	3.5
Jan. 3	Dolly		6.6	3.5	3.1
		Jan. 4 400			
Jan. 12	Dolly		5.6	1.7	3.9
		Jan. 18 300			
Dec. 20	Daisy		5.9	2.6	3.3
		Dec. 21 500			
Dec. 23	Daisy		5.9	2.6	3.3
Dec. 27	Daisy		5.7	2.1	3.6
Jan. 3	Daisy		6.7	3	3.7
		Jan. 11 300			
Dec. 20	Maude	800	7.1	2.8	4.3
Dec. 23	Maude		7.3	3	4.3
Dec. 27	Maude		6.3	2.3	4
Jan. 3	Maude		8.5	3.1	5.4
		Jan. 11 700			
Jan. 12	Maude		7.9	2.2	5.7
Dec. 20	Anita	700	5.7	2.2	3.5
Dec. 23	Antia		6	3	3
Dec. 27	Anita		6	3.2	2.8
Jan. 3	Anita		5.8	2.7	3.1
Jan. 12	Anita		6.1	1.5	4.6
		Jan. 18 400			
		Dec. 7 300			
Dec. 20	Shag		4.5	1.6	2.9
Dec. 23	Shag		4.6	1.5	3.1
Dec. 27	Shag		4.36	1.36	3
		Dec. 28 300			
Jan. 3	Shag		3.7	1.5	2.2
Jun. 12	Shag		3.8	0.7	3.1
		Jan. 15 300			

TABLE 4—Continued

Serum Proteins of Antitoxic Horse Serum

Albumin of Total Protein, %	Globulin of Total Protein, %	Nonprotein Constituents, %	Protein Quotient	Remarks
				Dec. 3, 1914, started immunization
50	50	1.1	1	
47	53	1.1	1	Dec. 23, 1915, 500 c.c. toxin
30	70	1.3	0.4	Dec. 27, 1915, 500 c.c. toxin
56	44	1.3	1.2	
30	70	1.3	0.4	Jan. 10, 1916, 500 c.c. toxin
44	56	1.1	0.8	May 6, 1915, started immunization
				Dec. 13, 500 c.c. of toxin
44	56	1	0.8	Dec. 23, 500 c.c. of toxin
36	64	1.3	0.56	Dec. 27, 500 c.c. of toxin
				Dec. 31, 500 c.c. of toxin
45	55	1.3	0.9	
39	61	0.96	0.6	Sept. 21, 1915, started immunization
				Dec. 15, 500 c.c. of toxin
41	59	0.77	0.7	
				Dec. 24, 500 c.c. of toxin
36	64	1	0.56	Dec. 27, 500 c.c. of toxin
				Dec. 31, 500 c.c. of toxin
36	64	1	0.56	Jan. 3, 500 c.c. of toxin
27	73	1.1	0.37	Jan. 12, 500 c.c. of toxin
38	62	1.1	0.6	Dec. 8, 1914, started immunization
				Dec. 13, 300 c.c. of toxin
50	50	1	1	Dec. 23, 200 c.c. of toxin
53	47	1.3	1	Dec. 27, 250 c.c. of toxin
				Dec. 31, 250 c.c. of toxin
46	54	1.2	0.8	Jan. 3, 250 c.c. of toxin
				Jan. 7, 300 c.c. of toxin
24	76	1.5	0.3	Jan. 10, 300 c.c. of toxin
35	65	1	0.5	Jan. 20, 1915, immunization started
				Dec. 10, 100 c.c. of toxin
32	68	0.87	0.47	Dec. 13, 150 c.c. of toxin
				Dec. 17, 200 c.c. of toxin
32	68	1	0.47	Dec. 20, 200 c.c. of toxin
				Dec. 29, 150 c.c. of toxin
				Jan. 3, 200 c.c. of toxin
40	60	1.1	0.66	Jan. 7, 200 c.c. of toxin
20	80	1.5	0.25	
				(Animal pregnant)

The percentage of total proteins showed very strikingly fluctuations. Although certain periods were associated with definite increase in the amount (March 31 and May 19), this increase was not a constant feature during the course of immunization. In this respect, our observations do not agree with those of previous workers.[1]

The considerable increase in the nonprotein constituents of the serum which occurred between May 1 and May 8 are difficult to explain, unless they were the result of some metabolic upset, although there is no definite evidence for this surmise.

In Table 4 will be found additional evidence in support of some of the points brought out in the more complete experiment. This table contains some determinations made at random intervals in 5 horses at various stages of diphtheria immunization. A careful analysis of the results demonstrates clearly the contention already made that the height of immunity stands in no direct relationship to the degree of globulin rise. A few specific illustrations may be cited. The protein quotient in horse Dolly was found to be 1 on December 21, and 0.4 on January 18, although the number of antitoxic units differed only by 100 on the 2 dates. Again, with very little change in the quotient in horse Daisy on December 21, and January 11, a considerable difference in the degree of immunity on these 2 dates was found to be present.

B. *Immunization of the Goat.*—Complete studies were made on the immune bodies and the serum proteins in 2 animals over a period of 4 months (Table 5 and Chart 2). The general deductions permissable from these experiments do not differ essentially from those given in connection with similar observations in the horse. It will be necessary merely to call attention to several details peculiar to these experiments. In neither goat was an initial injection of diphtheria antitoxin given. Immunization was started with small doses (0.001 c.c.), and reached toward the end of the period of immunization a maximum of 32 c.c. (200-300 M. L. D.).

The very definite absence of parallelism between antibodies and globulins is well shown in the study of both animals: Mephistopheles and Gretchen. It will be seen, by referring to the table and charts, that in the former the highest grade of immunity was attained on October 23. At this time the globulins constituted 76% of the total proteins (protein quotient 0.3). But already, on September 23, a similar rise in globulins had been observed, at a time when the animal showed a very slight grade of immunity (2 antitoxic units). Attention should, furthermore, be directed to the interesting observation that, on November 4, a still greater depression of the quotient (globulin rise) was effected by the injection of 10 c.c. of toxin and 20 c.c. of a 10-day old culture of diphtheria bacillus, although the number of antitoxic units had already fallen from about 12 to 4.

Similar discrepancy may be noted in the case of the 2nd goat, Gretchen (Table 5). Here it will be noted that, on October 2, the globulins constituted 92% of the total proteins and that the immunity corresponded to 3 antitoxic units; whereas 1 month later the immunity had increased (6 antitoxic units), while the percentage of globulins had fallen (74%).

The behavior of the total proteins in both goats is similar in every respect to that noted in the horse. Whereas the process of immunization causes fluctuations in the total proteins, we have failed to note any definite and constant increase throughout the period of observation.

[10] Butjagin, P. W.: Hyg. Rundschau, 1902, 12, p. 1193.   Joachim, J.: Arch. f. d. ges. Physiol., 1902, 93, p. 558.

C. *Immunization of the Rabbit.*—The immunization of rabbits with diphtheria or tetanus toxin is attended with great difficulties. This is more particularly true of diphtheria toxin. In this instance, successful immunizations can be carried out only if the toxin employed be attenuated. This was accomplished in our experiments by the addition of Lugol's solution to the toxin. In Table 6 and Chart 3, we have presented the detailed observations of 2 complete experiments with diphtheria toxin. In each experiment, the observations were extended over a period of 6 weeks, during which frequent determinations could be made of the degree of immunity developed and the concomitant alterations in the albumin-globulin ratio. These experiments illustrate in an unusual way the points which have already been emphasized in our experiments on the horse and goat, as well as in those previously recorded in connection with the influence exerted by bacterial toxins.[1]
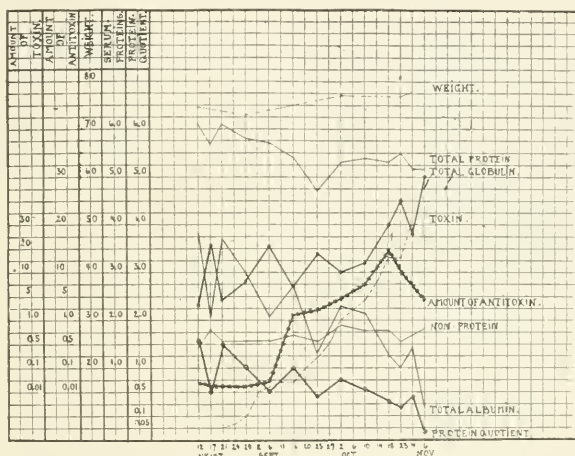
Chart 2.—Goat injected with diphtheria toxin.

The striking response of Rabbit 395 to inoculations of diphtheria toxin is well shown in Chart 3. It will be noted that, after the 2nd inoculation, an abrupt decline in the protein quotient (rise in globulins) occurred, and that this continued low until the end of the experiment, although the dose of toxin employed was not sufficient to develop within the animal any appreciable degree of antitoxic immunity. This animal possessed initially one fiftieth of an antitoxic unit which did not rise above one tenth of a unit during any period of the experiment. It must, therefore, be clear that the 2 responses of the animal are quite independent of one another, and that the inoculation of diphtheria toxin may produce this upset in the protein partition without at the same time giving rise to any appreciable grade of immunity.

The results of our observations on the 2nd animal (Rabbit 327) are essentially the same. These have been detailed in Table 6, and no additional comment is necessary.

TABLE 5

EFFECT OF THE INJECTION OF DIPHTHERIA TOXIN ON THE BLOOD PROTEINS IN THE GOAT "GRETCHEN"

Date	Amount of Toxin, C.c.	M. L. D.	Amount of Anti-toxin, Units	Weight, Pounds	Temperature	Total Protein, %	Total Albumin, %
Aug. 12	....	....	1/50	63½	37.9	5.5	1
Aug. 17	....	....	1/50	....	37.8	5.8	3.2
Aug 21	0.001	200	>1/50	64½	40.1	5.6	2.1
Aug. 24	0.002	200	......	....	39.1		
Aug. 29	0.005	200	>1/60	....	38.6	6.6	3.3
Sept. 2	0.01	200	......	....	39.5	...	...
Sept. 6	0.02	200	1/50	63¾	39.3	6.2	2.3
Sept. 11	0.04	200	......	....	38.7		
Sept. 16	0.04	1.+0.54 >200	½-1	....	38.6	5.9	2
Sept. 20	0.1	200	......	....	38.0		
Sept. 23	0.2	L+0.29	2	....	38.6	5.7	1
Sept. 27	0.5	L+0.29	......	....	38.5		
Oct. 2	1	L+0.425	3	....	38.9	5.4	0.4
Oct. 6	2	L+0.425	......	....	38.5		
Oct. 10	4	200	3-4	....	39	5.5	1
Oct. 14	8	L+0.54	......	....	39.3		
Oct. 18	16	200	4	....	39.5	5.7	2.1
Oct. 23	32	<100	3-4	61	37.8	5.8	1.7
Oct. 30	....	....	......	....	39.1	...	...
Nov. 4	8	300	6	57½	38.3	5.4	1.4
Nov. 6	....	....	5	....	37.9	5.2	0.1

TABLE 6

EFFECT OF THE INJECTION OF DIPHTHERIA TOXIN ON THE BLOOD PROTEINS IN THE RABBIT 327

Date	Amount of Toxin, C.c.	M. L. D.	Amount of Anti-toxin, Units	Weight, Gm.	Total Protein, %	Total Albumin, %	Total Globulin, %
Aug. 31	..	.....	<1/100	2900	5.3	4	1.3
Sept. 6	..	.....	<1/100	2850	5.5	3.5	2
Sept. 10	3	<100	<1/100	2800	5.8	3.2	2.6
Sept. 21	..	.....	<1/100	2700	5.7	2.7	3
Sept. 25	5	<100	1/100	2750	5.5	2	3.5
Sept. 28	.	.....	1/75-1/100	2675	5.8	2.6	3.2
Oct. 2	12	<100	1/75-1/100	2725	5.4	1.1	4.3
Oct. 9	..	.....	<1/75	2675	5.9	3.2	2.7
Oct. 11	5	200	1/75	2800			
Oct. 12	..	.....	1/50-1/75	2700	5.2	0.7	4.5

TABLE 5—*Continued*

EFEECT OF THE INJECTION OF DIPHTHERIA TOXIN ON THE BLOOD PROTEINS IN THE GOAT "GRETCHEN"

Total Globulin, %	Albumin of Total Protein, %	Globulin of Total Protein, %	Nonprotein Constituents, %	Protein Quotient	Remarks
4.5	20	80	1.4	0.25	
2.6	55	45	1.6	1.2	
3.5	40	60	1.6	0.6	No reaction
3.3	50	50	1.4	1	
.?.	..	..	...	...	Indigestion as a result of atony
3.9	37	63	1.4	0.6	of the rumen
3.9	34	66	1.4	0.51	
4.7	17	83	1.3	0.2	
5	8	92	1.5	0.08	Animal was pregnant
4.5	19	81	1.4	0.23	
3.6	36	64	1.5	0.56	
4.1	30	70	1.3	0.43	
...	..	..	...	....	Aborted 2 fetuses about 8-10 weeks old
4	26	74	1.5	0 35	Toxin was mixed with 30 c.c. of a 10-day culture of the diphtheria bacillus, Park and Williams, in Martin's broth
5.1	2	98	1.6		

TABLE 6—Continued

EFFECT OF THE INJECTION OF DIPHTHERIA TOXIN ON THE BLOOD PROTEINS IN THE RABBIT 327

Albumin of Total Protein, %	Globulin of Total Protein, %	Nonprotein Constituents, %	Protein Quotient	Remarks
75	25	2.1	3	
64	36	1.4	1.8	
55	45	1.4	1.2	Diphtheria toxin mixed with 1 c.c. of Lugol's solution, and kept at room temperature for 15 minutes
47	53	1.6	0.88	
34	66	1.8	0.5	Diphtheria toxin mixed with 2 c.c. of Lugol's solution; blood pressure low
44	56	1.6	0.8	
20	80	1.6	0.25	Diphtheria toxin mixed with 3 c.c. of Lugol's solution
54	46	1.5	1.1	
14	86	1.9	0.16	Died during the afternoon; hemorrhagic gastro-enteritis; focal necrosis of the liver; hemorrhages in the mesentery and cortex of suprarenals; acute nephritis

D. *Immunization of the Dog.*—In the description of the methods employed in our study, brief mention was made of the great susceptibility of the dog to toxin inoculations. Such inoculations frequently result in necrosis and sloughing at the seat of injection. In a previous paper, it has been shown that such secondary infections, in themselves, will produce a striking upset in the partition of the protein fractions, which at times may amount to a complete inversion of the formula. To overcome this complication, several modifications of the technic used in the case of the other animals experimented on were tried. An attempt was first made to lessen the initial response of the animal by the inoculation of balanced mixtures of diphtheria toxin and antitoxin. But such mixtures were not found to give the desired effect. Necroses and sloughs still resulted. Better success was obtained by initial inoculations of diphtheria antitoxin. As soon as a basic immunity was established, hyperimmunization could be accomplished without the production of sloughs at the site of inoculation.
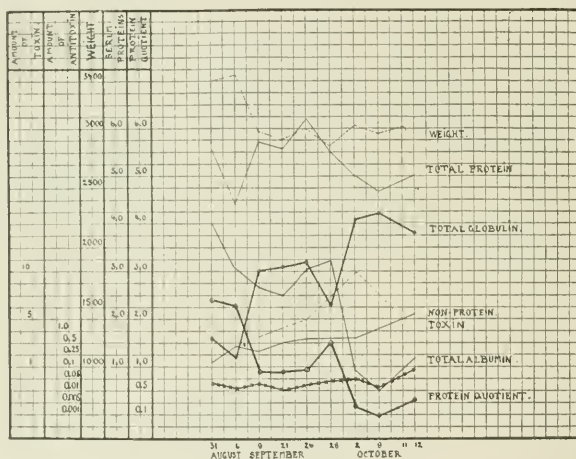
Chart 3.—Rabbit injected with diphtheria toxin.

Of the 6 dogs experimented on, we wish to present the results obtained in 2 animals only, because these are more complete and are representative of all the others. The detailed observations are given in Table 7 (Dog 641) and Chart 4 (Dog 640). But for purposes of clearness, it may be well to call attention to a few of the interesting features of these experiments.

The immediate effect which an inoculation of toxin may have on the albumin-globulin ratio is well shown in Table 7 (Dog 641). In this animal, notwithstanding the protective injection of 1 large dose of antitoxin, great fluctuations in the protein quotient were observed, due to the immediate rise in the globulins following the repeated inoculations. Such periods of globulin increase, as can be readily seen from the table, were not attended by a corresponding rise in the number of antitoxic units. These continued low for 2 months after the beginning of the experiment, when they increased to a level of 14-15 units, and then showed a tendency to decline, due to the lower toxicity of the prepa-

ration employed at this time.* The figures of the table show clearly that periods of increased immunity do not parallel periods associated with an increase in the globulin content. Thus, on July 1 and 24, this animal showed a globulin content of about 92-93%, with an antibody content of $\frac{1}{3}$-$\frac{1}{2}$ of an antitoxic unit, whereas, on August 19, with a maximum number of antitoxic units 14-15, the globulin percentage only reached 53. A similar lack of parallelism will be seen in the results of Dog 640, graphically presented in Chart 4. This shows in an unusual way the gradual rise in the antibody curve without a parallel rise in the curve of the protein quotient. Such fluctuations as were noted are for the most part transitory. The failure to obtain any higher grade of immunity, despite the increase in the dosage of the toxin, must be attributed to the low toxicity of the preparation available at this time.
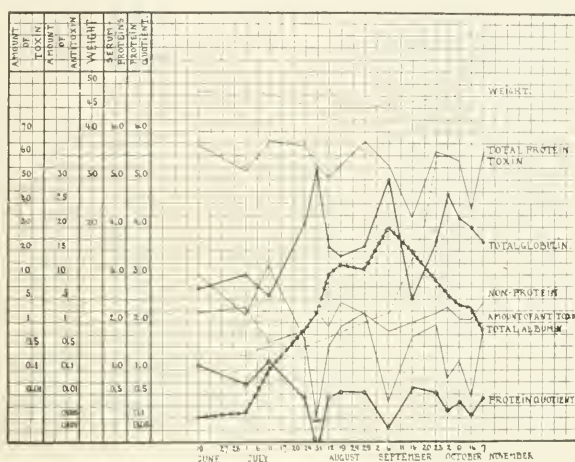
Chart 4.—Dog injected with diphtheria toxin.

The chart shows, furthermore, 2 additional features worthy of emphasis. It will be noted, in the first place, that the increase in globulins occurs, for the most part, at the expense of the albumin fraction, so that the latter curve is almost a mirror-image of the former, a relationship of the 2 fractions which has also been noted in bacterial infection and immunity; secondly, attention should be called to the considerable fluctuations in the total proteins of the blood serum without any constant and persistent increase in their percentage during the entire period of immunization.

II. IMMUNIZATION WITH TETANUS TOXIN

The literature contains only an occasional observation of the influence of tetanus toxin on the blood proteins. Because of the interesting results we obtained by the use of diphtheria toxin, we thought it worth while to extend our studies in this direction, although our experiments

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* Martin L. (Ann. de l'Inst. Pasteur, 1898, 12, p. 26) has shown that a low antitoxic serum will result, following the injection of a weak subsequent to inoculations of a strong one.

TABLE 7

EFFECT OF THE INJECTION OF DIPHTHERIA TOXIN ON THE BLOOD PROTEINS IN THE DOG
Dog 641

Date	Amount of Toxin, C.c.	M. L. D.	Amount of Anti-toxin, Units	Weight, Pounds	Tempera-ture	Total Protein, %	Total Albumin, %
June 19	...	...	>1/150	40½	38.2	5.7	2.5
June 27	...	...	......	39½	38.8	...	...
June 28	0.3	200	......	....	39.1	...	...
July 1	0.3	200	1/20	....	38.6	5.2	0.43
July 6	0.3	200	......	....	38.3		
July 11	0.5	200	1/5	....	39.0	6.2	3.2
July 17	0.6	200	......	....	38.3		
July 20	...	...	......	42	39.6	...	...
July 24	...	...	1/2	40½	38.3	6	0.5
July 31	...	...	1–2	....	38.1	5.9	1.3
Aug. 12	0.7	200	......	41½	38.2	5.5	2.5
Aug. 19	1	200	14–15	42½	38.1	5.5	2.6
Aug. 24	1.5	200	......	41¾	38.3		
Aug. 29	2.5	200	12	43	38.3	5.9	2.3
Sept. 2	5	150–200	......	....	37.9		
Sept. 6	10	200	10	....	39.0	5.4	0.4
Sept. 11	20	<100	......	....	38.1		
Sept. 16	40	<100	15	....	38.2	6.5	3.5
Sept. 20	80	<100					
Sept. 23	....	...	10	....	....	6	2.1
Oct. ?	30	<200	6–8	43	38.2	5.1	0.1
Oct. 9	....	...	6	....	38.1	6	4
Oct. 16	....	...	5	....	38.1	5.7	0.9
Nov. 7	....	...	2	43¾	....	5.6	1.4

TABLE 8

SERUM PROTEINS OF ANTITOXIC HORSE SERUM
TETANUS

Date	Number of Horse	Amount of Antitoxin, Units	Total Protein, %	Total Albumin, %	Total Globulin, %
Jan. 17	12 (Op. 216)	500	7.3	2.7	4.6
Jan. 17	13 (Op. 217)	200	5.9	2.3	3.6
Jan. 23	15 (Op. 218)	125	5.7	2.7	3
Feb. 7	(Op. 223)	100	5.7	1.8	3.9
Feb. 7	(Op. 224)	602	7.2	2.7	4.5
Feb. 7	(Op. 225)	250	6.9	3	3.9
Feb. 7	(Op. 226)	400	5.9	2.3	3.6
Feb. 7	(Op. 227)	200	5.7	2.3	3.4
Feb. 10	(Op. 228)	909	7.9	2.8	5.1

TABLE 7—*Continued*

Effect of the Injection of Diphtheria Toxin on the Blood Proteins in the Dog 641

Total Globulin, %	Albumin of Total Protein, %	Globulin of Total Protein, %	Nonprotein Constituents, %	Protein Quotients	Remarks
3.2	50	50	2.6	1	
...	..	..	...	...	6000 units diphtheria antitoxin subeutaneously
...	..	..	...	...	No clinical symptoms
4.8	7	93	2.3	0.07	No symptoms
3	51	49	1.8	1	Swelling at seat of injection
...	..	..	...	...	Slight intoxication resulting from accidental feeding of liver tissue; considerable swelling at seat of last injection; warm and tender
5.5	8	92	1.6	0.08	At seat of last inoculation, a dark, necrotic, dry slough 5½ × 5 cm. diameter
4.6	22	78	2.2	6.3	Necrotic area well demarcated; removed slough, and treated granulated wound with methylene blue
3	45	55	1.9	0.8	Wound 1½ × 1½ cm. diameter was granulating well
2.9	47	53	2.3	0.88	At seat of last injection, swelling size of a hazel nut
3.6	40	60	2	0.6	
5	6	94	2	0.06	
3	46	54	2.1	0.8	
3.9	35	65	2	0.5	
5	2	98	2	0.02	
2	60	40	2	1.5	
4.8	16	84	1.9	0.2	
4.2	25	75	2	0.3	

TABLE 8—*Continued*

Serum Proteins of Antitoxic Horse Serum
Tetanus

Albumin of Total Protein, %	Globulin of Total Protein, %	Nonprotein Constituents, %	Protein Quotient	Remarks
37	63	1.2	0.6	
39	61	1.4	0.65	Maximum unitage
47	53	1.4	0.88	
57	69	1.4	0.45	Decline of antitoxin production
37	63	1.4	0.6	Decline of antitoxin production
43	57	1.3	0.75	Maximum unitage
39	61	1.5	0.6	
40	60	1.4	0.66	
54	46	1.1	1.1	Maximum unitage

with the use of tetanus toxin are not so complete. Besides a series of determinations on the albumin-globulin ratio of 9 horses studied during various stages of tetanus immunization, we have carried out a number of systematic experiments on rabbits. The general methods employed are analogous to those described in connection with the use of diphtheria toxin. The use of so sensitive an animal as the rabbit necessitated an attenuation of the toxin with Lugol's solution and a careful grading of dosage, so as to make a more prolonged study of the animals possible. In the beginning of this series, we failed to attenuate the toxin sufficiently so that the animals succumbed after a sho period with symptoms of tetanus intoxication. The intervention o uch symptoms, although not anticipated, proved to be of extreme i est,
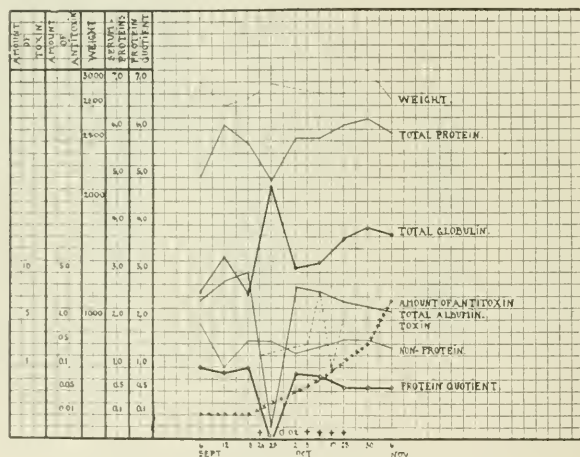
Chart 5.—Rabbit injected with tetanus toxin.

for as will appear (Table 10), this mishap threw some light on the behavior of the serum proteins during the development of this particular intoxication.

In Table 8, we have recorded the results of determinations made on 9 horses, at varying intervals, during immunization with tetanus toxin. The figures support in an unusual way the conclusions to which we have arrived from previous experiments. At the time the serum proteins were quantified, we as yet had no definite knowledge of the degree of immunity present in the animals studied. A comparison of the figures obtained for the antitoxic units with those for the globulin content permit of no doubt concerning the justness of our contention that there can be no relationship between the 2 phenomena.

A few instances in support of this fact will suffice. In the table it will be noted that the horse (operation-specimen No. 228) which developed the highest grade of immunity (909 antitoxic units) showed on the day the proteins were studied a globulin percentage of 46; whereas, in the horse (Op. 218) which developed the lowest immunity of this series, we observed a slightly higher globulin content.

In order to obtain additional evidence for or against this deduction, however, we carried out 2 complete immunization experiments on rabbits (Table 9 and Chart 5). Reference to the general procedure used has been made, and attention need only be directed to a few of the detailed results. These findings (Chart 5) are presented in a very striking manner for 1 of the animals (Rabbit 714). Only at 1 period during the experiment did the globulin fraction rise precipitously (fall in protein quotient). This occurred on September 28, following the injection of 1.5 c.c. of tetanus toxin. Four days later the quotient showed a tendency to return to a normal level and the curve now continued along a parallel course despite the very definite increase in this animal's immune state, as indicated by the great rise in the number of antitoxic units (2-5 units). A similar result was obtained in the 2nd animal (Rabbit 715, Table 9), but, in this instance, the striking rise in the globulin percentage was somewhat retarded, and occurred only after the 2nd and 3rd injections of tetanus toxin. Notwithstanding the gradual increase in the animal's immunity, the protein quotient tended to return to its initial normal level. Such an observation has already been recorded in connection with some experiments previously published on the effect of bacterial toxins on changes in the blood protein fractions.

Interesting in this connection are the 2 observations which we made on the influence of tetanus intoxication on the blood proteins. As previously stated, these results were quite incidental and not anticipated, but they afford data of great comparative value. The attenuation of the toxin was apparently not sufficient to prevent the appearance of toxic symptoms, for both animals died on the 7th day with typical signs of tetanus: opisthotonus trismus, salivation, and clonic and tonic spasms. In spite of this mode of death, no change occurred in the albumin-globulin ratio. It is somewhat difficult to harmonize this finding with those previously recorded in other forms of intoxication. It has been found, for instance, that animals developing toxic symptoms after overwhelming inoculations of bacterial toxins[1] or following the absorption of loop proteose[17] invariably show an increase in the globulin content. The animals dying of tetanus intoxication, however, showed no alteration in the quotient. The only possible explanation for this apparent discrepancy, it seems to us, must be sought in the nature of the tetanus toxin. The latter is, as we know, primarily a neurotoxin, with an almost specific affinity for nervous tissue; whereas the other toxic substances produce very striking alterations in body tissues in general. Since it has become clear that alterations in the albumin-globulin ratio stand in intimate relationship to a general metabolic disturbance associated with much tissue destruction, it is not surprising to find an absence of fluctuations in the protein quotient during the action of a substance possessing, for the most part, a neurotoxic action.

[17] Hurwitz, S. H., and Whipple, G. H.: Jour. Exper. Med., 1917, 25, p. 231.

III. IMMUNIZATION WITH BOTULISM TOXIN

Through the kindness of Dr. E. C. Dickson, some potent toxin of
B. botulinus was placed at our disposal. The study of immune reac-
tions with this soluble toxin appealed to us as of especial interest from
a comparative viewpoint, because it has been established that the local
and constitutional reaction following the injection of this toxin differ
in many important respects from those met with after inoculations of
diphtheria and tetanus toxin. Unlike the latter 2 toxins, botulism
toxin does not produce so marked a constitutional and temperature
reaction, and in other respects is quite different in its manifestations.
Because of these points of difference, it seemed worth while to deter-

TABLE 9

EFFECT OF THE INJECTION OF TETANUS TOXIN ON THE BLOOD PROTEINS IN THE RABBIT

Date	Amount of Toxin, C.c.	M. L. D.	Amount of Anti- toxin, Units	Weight, Gm.	Total Protein, %	Total Albumin, %	Total Globulin, %
Sept. 13	..	...	<1/20	2525	5.7	3.9	1.8
Sept. 1s	..	...	<1/20	2625	5.7	3.8	1.9
Sept. 26	1	100	......	2600	...	...	...
Sept. 2S	..	...	......	2700	5.6	3.1	2.5
Oct. 2	..	...	<1/20	2700	6	4.2	1.8
Oct. 3	5	100	......	2675	...	...	...
Oct. 9	12	100	<1/10	2600	5.7	2	3.7
Oct. 16	..	...	<1/10	2625	6.2	1	5.2
Oct. 17	1	1000	......	2700			
Oct. 23	5	1000	>1/5	2520	6.1	2.S	3.3
Oct. 31	..	...	>1/5	2850	6.1	2.S	3.3
Nov. 6	..	...	1+	2660	6.5	4.3	2.2

min in what manner inoculations of the toxin of botulism affected the
partition of the blood proteins, and how these alterations were related
to the grade of immunity developed.

Our experiments were carried out on dogs. It is commonly stated
that this animal is less susceptible to botulism toxin than are the smaller
laboratory animals, rabbits and guinea-pigs. But we have found in
confirmation of the work of Dickson that this is not always the case,
for the sample of toxin employed by us proved to be quite potent in its
action, as evidenced by the death of 1 of the animals experimented on,
and by the development of a fairly high grade of immunity in the 2nd
dog. Because of the uniqueness of the observations with this toxin, a
more detailed citation of the experiments carried out may be of interest.

EXPERIMENT 1.—*Death from Botulism Toxin.*—A black mongrel male weighing 35 pounds was inoculated subcutaneously on December 20, with 0.05 c.c. of toxin. No reaction occurred. On December 22, 0.1 c.c. toxin was inoculated. This was followed by the injection of 0.2 c.c. toxin on December 26, and 0.5 c.c. on December 30. Two days later the animal was prostrate. There was salivation, constipation, conjunctivitis, paresis of the hind legs, ataxia, and pharyngeal palsy. The animal vomited frothy bile and mucus, showed excessive salivation and lacrimation, and died at 1:30 p. m.

. *Autopsy.*—The abdominal cavity was dry. A small amount of blood-stained fluid was found in both pleural cavities, a few drops of fluid in the pericardial sac.

The spleen was of normal size, but showed soft dark infarct. The kidneys were normal in size and appearance.

The jejunum was dry and revealed bile-stained contents. The ileum, colon, and rectum were very dry, and revealed poorly digested contents.

TABLE 9—*Continued*

EFFECT OF THE INJECTION OF TETANUS TOXIN ON THE BLOOD PROTEINS IN THE RABBIT

Albumin of Total Protein, %	Globulin of Total Protein, %	Nonprotein Constituents, %	Protein Quotient	Remarks
68	32	1.7	2.1	
66	34	1.6	1.9	
..	..	...	...	2 c.c. of tetanus toxin mixed with 2 c.c. of Lugol's solution, and kept at room temperature for 45 minutes
55	45	1.9	1.2	
70	30	1.6	2.3	
..	..	...	...	5 c.c. of toxin mixed with 2 c.c. of Lugol's solution
35	65	1.4	0.5	12 c.c. of tetanus toxin mixed with 2 c.c. of Lugol's solution
17	83	1.6	0.2	
45	55	1.4	0.8	
45	55	1.4	0.8	
66	44	1.2	1.6	

The liver was enlarged, engorged with blood, and revealed a granular surface. The gallbladder was distended with deep greenish-yellow bile. The mucous membrane was covered with a thick layer of mucin; numerous petechiae were observed.

The stomach contained frothy, bile-stained mucus. The mucous membrane was uniformly bile-colored. The fundus region was deep red.

The heart was flabby; the endocardium and valves were normal.

The bronchial mediastinal lymph nodes were enlarged.

The lung revealed a small thrombus in the pulmonary artery, and an infarct surrounded by compensatory emphysema. The middle lobe was pneumonic, and showed fresh fibrinous exudate on the pleura. In the right principal lobe, there was an infarct the size of a large pea; numerous similar infarcts were found in the left anterior heart lobe and principal lobe. The trachea and bronchi contained blood-stained froth.

The base of the brain was distinctly injected, as was also the dura of the spinal cord.

Microscopic study showed multiple thrombi in lung, spleen, and base of brain.

The fluctuations which occurred in the serum-protein fractions during the course of immunization and the number of antitoxic units produced are presented in detail in Table 10.

EXPERIMENT 2.—*Successful Immunization with Botulism Toxin.*—A mongrel male dog weighing 40½ pounds was inoculated on January 18, with 0.1 c.c. of toxin (M. L. D. 2000). Two subsequent inoculations of 0.2 c.c. of toxin were given on January 25 and 30. On February 5, the animal showed a slight conjunctivitis and rhinitis. Three days later (February 8), animal received 0.5 c.c. toxin (M. L. D. 1000) and on February 14, 0.8 c.c. of the same toxin was given. On February 20, animal received 1 c.c. of toxin (M. L. D. 500), twice

TABLE 10

EFFECT OF THE INJECTION OF BOTULISM TOXIN ON THE BLOOD PROTEINS IN THE DOG

Date	Amount of Toxin, C.c.	M. L. D.	Amount of Antitoxin, Units	Weight, Pounds	Temperature	Total Protein, %	Total Albumin, %
Dec. 18	....	....	<1/20	35	39.9	3.9	2.1
Dec. 19	....	....	......	..	38.1	4.8	2.8
Dec. 20	0.05	2000	......	..	39.4	5.3	2.8
Dec. 22	0.1	2000	......	35	39.1	5.3	3.3
Dec. 26	0.2	2000	......	..	39.3	5.3	3.6
Dec. 28	...	....	......	..	37.8	5.4	3.8
Dec. 30	0.5	2000	......	..	38.1	5.4	2.3
Jan. 2	...	....	......	31.5	39.1	5.1	2.8
Jan. 4	...	....	<1/20	31.5	38.7	5.8	3.2
Jan. 5	. .	....	......	31	40.5	5.9	3.3

this dose 4 days later, and 4 c.c. of the same toxin 8 days later. Two c.c. of a stronger toxin (M. L. D. 2000) was administered on March 7. On March 9, animal was found sick, and showed a subnormal temperature. The next 2 injections of 4 c.c. and 8 c.c., on March 14 and 17, respectively, were well tolerated. On March 26, a final injection of 15 c.c. of toxin of a strength greater than 2000 M. L. D. produced vomiting and constipation on the day following. With the exception of a moderate loss in weight during the period of immunization, the animal showed no other untoward symptoms, beside those already mentioned.

Daily specimens of blood were obtained for a determination of the degree of immunity present and for a quantification of the serum proteins. The interesting changes which this dog showed in the albumin-globulin ratio following the toxin injections are graphically presented in Chart 6.

The most striking feature of both the experiments with botulism toxin, and more especially of the latter, is the character of the immediate response of the animal to the inoculations. Unlike the reaction observed for other toxins, botulism toxin causes a depression of the

globulin content, with a corresponding rise in the albumin fraction. In Experiment 2, this first becomes noticeable after the 3rd inoculation (January 30), when the globulins begin to fluctuate gradually downward. The protein quotient shows marked fluctuations with a tendency to rise tremendously (fall in globulins) about 24-48 hours after each inoculation, reaching its maximum height about 2 months after the onset of the experiment. Only after the 3 last inoculations of massive doses of a strong toxin did the protein quotient tend to remain more or less permanently depressed. Careful analysis of the curve clearly

TABLE 10—*Continued*

EFFECT OF THE INJECTION OF BOTULISM TOXIN ON THE BLOOD PROTEINS IN THE DOG

Total Globulin, %	Albumin of Total Protein, %	Globulin of Total Protein, %	Nonprotein Constituents, %	Protein Quotients	Remarks
1.8	55	45	2.6	1.2	By mistake vaccinated against distemper on Dec. 17
2	58	42	2	1.3	
2.5	53	47	1.8	1.1	
2	63	37	1.5	1.7	
1.7	68	32	1.4	2.1	
1.6	71	29	1.4	2.4	
3.1	43	57	1.3	0.75	
2.3	55	45	2.7	1.2	Dog unable to rise, constipated, salivation, and conjunctivitis, pupils wide open, posterior paresis
2.6	56	44	2.2	1.2	Spastic posterior paresis, pharynx paralysis, accelerated pulse, 126; signs of ataxia
2.6	56	44	2.3	1.2	Vomited frothy bile and mucus, excessive salivation and lacrimation; died at 1:30 p. m., blood collected at 10 a. m. Necropsy: thrombosis of pulmonary arteries and veins

shows that the increase in the albumin fraction occurred at the expense of the globulin fraction, for the 2 curves are almost mirror-images of one another.

The total proteins fluctuated considerably during the experiment, and at times showed a slight tendency to rise, but no definite and persistent increase was noted for the whole period of immunization.

DISCUSSION

The studies of a number of workers and our own have shown with considerable certainty that an increase in serum globulins occurs with great constancy at one period or another during the immunization of an animal with a soluble toxin. But the question of the possible significance of this increase in relation to immunity and to the chemical nature of the protective substances formed has called forth numerous

studies and conflicting deductions. The evidence gained from our experiments with soluble toxins supports the contentions which we presented in a previous paper on the relation between blood globulins and bacterial infection and immunity. In general, the observations permit of the conclusion that no constant relation exists between the extent of serum globulin increase and the antitoxic content of the serum. The reasons for this deduction have been discussed in some detail in connection with the citation of the separate experiments, and for this reason, we believe that any additional comment is not needed. It may be more desirable to touch on the possible influence which certain experimental factors may have in giving rise to this increase in the globulins and to present certain tentative suggestions concerning other causes which may explain the observations presented.

The use of a variety of animals, as well as of a number of different toxins, has brought out several interesting points relative to animal susceptibility and the response to the toxin inoculations. It has been found, for instance, that in the less susceptible animal, the toxin must reach a certain threshold before a marked and constant depression of the protein quotient occurs. Thus, in the horse and goat, immunization must be continued over a longer period of time or with more potent toxins before any appreciable changes are produced; whereas in the dog small amounts of some of the toxins will rapidly bring about a striking alteration in the albumin-globulin ratio. Thus it would seem that something in the nature of a cumulative action or a summation of effects is needed to explain the delayed reaction observed in the less susceptible animals. Some such inference may serve also to explain the very unique observations made in connection with botulism toxin. It will be recalled that this toxin evokes a rise in the albumin fraction instead of the usual increase in the globulin fraction which may not occur until late in the experiment. The meagerness of our knowledge concerning the nature of botulism toxin does not justify extensive speculation, but it may not be too far afield to entertain the possibility that this toxin contains more than 1 toxic substance, and that 1 of these gives rise to an increase in the protein quotient; whereas the other causes a depression of it. Inasmuch as a depression of the quotient occurs after the inoculation of the majority of toxins, we may assume that the cause of this is attributable to its nonspecific component, which must reach a certain concentration before it becomes effective. On the other hand, the immediate and constant rise in the albumin fraction following the injections even of small doses of botulism toxin favors the assumption that this is due to the activity of
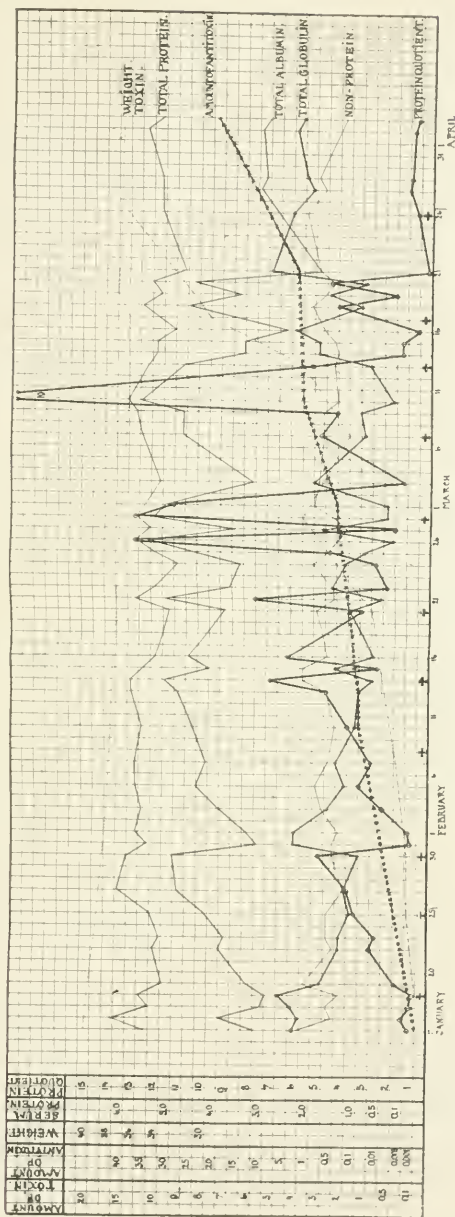
Chart 6.—Dog injected with botulism toxin.

a very active specific component which behaves in this characteristic way. Such speculation may gain some support from the study of other toxins which behave in this peculiar way. Thus far, however, this is an isolated observation.   ,

It may be further noted that the animals inoculated with botulism toxin showed no constitutional reaction and responded by the production of antibodies in high concentration, although this response was associated in the beginning with a rapid rise in the albumin fraction. This is in striking contrast to the findings in those animals which reacted severely to the inoculations. As has been pointed out in another connection, such animals usually show a high globulin content. It has been shown by von Ermengen that the dog is not very susceptible to inoculation of botulism toxin, but Dickson has shown that the toxic substances produced by the California strain may be fatal in comparatively small doses, when inoculated into the dog.

From a large number of observations, we have become convinced that the serum globulin increase does not represent the accumulation of protective substances as Mellanby[18] and others have assumed. It would appear rather that other factors are responsible for the changes in the protein partition observed.

Excessive bleedings and the injection of large amounts of culture mediums are in themselves not responsible for the alteration in the albumin-globulin ratio.

To overcome the possible disturbing influence of excessive bleedings for serum-production, Gibson and Banzhaf bled their animals only after maximum antitoxic and protein changes had occurred, so they conclude that the variations observed during the period of increasing antitoxic potency are not influenced by severe hemorrhage. Furthermore, the possible influence of this factor in the production of the changes noted can be excluded in the smaller animals like the dog or rabbit, where the individual bleedings were small (10 c.c. for dog and 5 c.c. for rabbit).

Still another factor has been suggested as the possible cause of the rise in globulins, namely, the injection of large quantities of culture mediums together with the toxin. This point has been especially studied by Ledingham[6] during the immunization of the horse with diphtheria toxin. This worker was able to show conclusively that the inoculation of as large a quantity as 800 c.c. of bouillon into a susceptible horse did not produce any appreciable change in the globulin

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[18] Proc. Royal Soc., Series B, 1908, 70, p. 399.

percentage. Our own studies have shown that the injection of about 10 c.c. of bouillon into the dog gives rise to only normal fluctuations in the protein fractions, whereas a similar quantity of broth-containing toxin causes a very striking alteration of the ratio. It is, therefore, unlikely that either excessive hemorrhage or the injection of large quantities of culture medium can explain the changes observed.

Whatever the ultimate cause or causes of these phenomena may be, there is good reason to believe that the upset in the delicate protein balance of the blood is 1 manifestation of a disturbed metabolism resulting from the toxin inoculations. The reasons for assuming the existence of a metabolic disorder in the animals studied have been given and discussed in previous papers. At that time we ventured the suggestion that the heaping up of blood globulins might be the result of the more rapid formation of this molecule. We have come to believe, however, that such speculation is not permissible at the present time because of the incompleteness of our knowledge concerning the chemical relationship of the albumin and globulin fractions. It is fair to state, however, that the work[19] which has been done thus far strongly suggests that the conversion of the one fraction into the other, or the change of one form of globulin into another is brought about by an alteration of the physico-chemical properties of the blood serum, properties on which, in all probability, depend the differentiation of the various protein fractions by "salting out methods." It may, therefore, not be too venturesome to suggest that the alteration in the normal albumin-globulin ratio which has been observed during immunization may be attributable to change in the colloidal properties of the serum, which may in great measure determine its reaction to the various precipitating salts.

CONCLUSIONS

The percentage of serum globulins increases markedly during the course of immunization with diphtheria, tetanus, and botulism toxin. In the case of botulism toxin, however, there is first an initial rise in the albumin fraction.

No constant relationship is demonstrable between the percentage increase in the serum globulin and the antitoxic potency of the serum.

The rise in globulins may be 1 manifestation of an upset in the delicate protein balance of the blood, resulting from the disturbed metabolism following the toxin inoculations.

[19] Berg, W. N.: Jour. Agric. Research, 1917, 8, p. 449.

FURTHER STUDIES ON THE EFFECTS OF THE ROENTGEN RAY ON ANTIBODY-PRODUCTION

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In a previous paper[1] are given the results of observations on the influence of the roentgen ray on the production of lysin for sheep corpuscles in the white rat. It was found that prolonged exposure to roentgen ray, about the time the antigen was injected, markedly reduced the production of lysin, due, it was assumed, to destructive action on the lymphatic tissues, the spleen, and the marrow. In order to test the effect of the ray on antibody-production after it is well under way, that is, a few days after the introduction of the antigen, it proved advisable to use animals that would stand repeated bleedings better than the rat. The purpose now is to present briefly the results of experiments of this and like nature on the dog and the rabbit.

In these experiments, the only variation in the treatment with roentgen ray was in the length of exposure. The radiation was always made with the Coolidge tube, the focal distance always 8 inches, the current 5 to 6 milliamperes, spark-gap 8 inches. The exposures were made in the roentgen-ray laboratory of the Presbyterian Hospital, under the direction of Dr. J. W. Rowntree, to whom I am indebted very much. Young animals were used, dogs a few weeks old, and rabbits weighing about 1000 gm.

As a rule 2 exposures were made, a major followed by one one-fourth as long the next day immediately before the injection of the antigen. In the dogs, the antigen, 1 c.c. 10% suspension of rat or goat blood per kilo of weight of dog, was injected intravenously; in the rabbit, 25 c.c. of sheep blood were injected intraperitoneally. Only one injection of antigen was given. In the dog, the antibodies measured were the agglutinin and opsonin for rat corpuscles and the lysin for goat corpuscles; in the rabbits, the titers of the lysin for sheep corpuscles and of the precipitin for sheep blood proteins were determined. The figures in the tables give the highest active dilutions of the serum in the case of the agglutinin, opsonin and lysin tests, and the highest dilution of sheep blood, in which the rabbit serum produced precipitate by the ring or contact method in the case of the precipitin tests. The details of making the tests are described in previous articles.[2]

[1] Jour. Infect. Dis., 1915, 17, p. 415.

[2] Ibid., 1910, 7, p. 319; 1914, 14, p. 403; 1916, 19, p. 69.

EFFECT OF ROENTGEN RAY AT THE BEGINNING OF ANTIBODY-PRODUCTION

The results of new experiments on the action of roentgen ray at the beginning of antibody-production are illustrated in the instances from various experiments brought together in Tables 1 and 2. Table 1 shows that in dogs (Dogs 2 and 5) treated in the manner I have indicated, a marked reduction in the output of antibodies is obtained by a 10-minute exposure followed the next day by a 2½-minute exposure (approximately 37½ Kienbach units). In dogs so treated, there

TABLE 1

EFFECT OF THE ROENTGEN RAY ON THE DEVELOPMENT OF ANTIBODIES IN THE DOG

Days After Infection of Antigen	Injected With Rat Blood			Injected With Goat Blood			Remarks
	Dog 1; Roentgen Ray 2½ min., the Next Day 40 sec.	Dog 2; Roentgen Ray 10 min., the Next Day 2½ min.	Dog 3; Roentgen Ray 20 min., the Next Day 5 min.	Dog 4; Roentgen Ray 2½ min., the Next Day 40 sec.	Dog 5; Roentgen Ray 10 min., the Next Day 2½ min.	Dog 6; Roentgen Ray 20 min., the Next Day 5 min.	
2	24			96			
4	24	96	0	192	192	48	Leukocytes: Dog 3-3600, Dog 6-2200
6	192	768	0	384	768	96	Leukocytes: Dog 3-3000, Dog 6-2400
8	1536	768	0	6144	1536	96	Leukocytes: Dog 3-1800, Dog 6-3000
10	3072	384	24	6144	1536	96	
12	3072	384	96	6144	1536	96	Leukocytes: Dog 3-1500, Dog 6-2100
14	3072	384		1536	768		
16	3072		96	1536		96	Marked Roentgen-ray burns in Dogs 5 and 6
18		142			384		
20	1536			768			
22	768	192		768	24		
24							
26	96			96			
28							
30	96			96			

may be only little apparent disturbance of the general health and no great change in the leukocytes in the peripheral blood; an occasional animal succumbs, however, and there may be then marked roentgen-ray effects. Dogs 3 and 6, Table 1, illustrate that treatment with roentgen ray for 20 minutes and again for 5 minutes the next day (75 Kienbach units) may restrain practically completely all production of antibodies in response to antigen injected immediately after the 2d exposure. In such animals, there is usually reduction in the leukocytes in the blood with relative decrease in the granular and relative increase in the nongranular forms; severe burns may develop, and

the thymus, spleen, and marrow show evidences of cell destruction. Dogs 1 and 4, Table 1, appear not to have suffered any loss of power to elaborate antibodies, as the result of exposures to roentgen ray for $2\frac{1}{2}$ minutes and then for 40 seconds ($9\frac{1}{2}$ Kienbach units), the course and quantity of antibodies produced by these animals correspond to normal standards.

The rabbit is less satisfactory to work with than the dog because of the much higher death rate and the much greater individual variation in response to antigen. As shown in Table 2, however, results are obtained which correspond to those in the white rat and the dog.

TABLE 2

EFFECT OF THE ROENTGEN RAY ON THE DEVELOPMENT OF ANTIBODIES IN THE RABBIT

Days After Injection of Sheep Blood	Rabbit 1; Roentgen Ray 10 min., the Next Day 2½ min.		Rabbit 2; Roentgen Ray 5 min., the Next Day 1½ min.		Rabbit 3; Roentgen Ray 2 min., the Next Day ½ min.		Control		Remarks
	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin	
5	512	0	2048	0	4096	0	768	0	In Rabbit 1, the
8	2048	400	8192	800	8192	800	6144	800	leukocytes
10	6144	400	3072	400	8192	6400	12288	3200	came down to
12	3072	1200	6144	800	24576	12800	12288	6400	1400 with a
14	1536	1200	3072	800	12288	6400	6144	12800	late relative
16	1536	800	1536	800	6144	6400	3072	12800	decrease in
19	1536	400	3072	800	6144	4800	3072	12800	granular and
22	1536	800	3072	800	6144	4800	3072	12800	relative in-
26	1536	200	1536	400	6144	1600	3072	12800	crease in non-
30	768	200	768	400	1536	1600	3072	3200	granular leu-
35	1536	400	768	800	1536	1600	3072	3200	kocytes. In
41	768	200	768	400	3072	1600	1536	1600	Rabbits 2 and
45	1536	800	768	400	3072	1600	1536	1600	3, no definite
51	768	200	384	400	3072	1600	1536	1600	change in
62	192	400	192	400	1536	1600	1536	800	leukocytes
89	192	400	384	200	1536	400	768	200	

EFFECT OF THE ROENTGEN RAY AT OR NEAR THE HEIGHT OF ANTIBODY-PRODUCTION

In this series of animals, in which the manufacture of antibodies was well under way, were exposed to roentgen ray and an effort made to determine whether this would affect the subsequent course of the antibodies in the blood. Table 3, which is representative of several experiments of this kind, appears to show that a 20-minute exposure on the 7th day after the injection of the antigen had no demonstrable effect, as the antibodies continue to run their course precisely as in dogs injected in the same way, but not put under the influence of the ray. Even when combined with splenectomy exposure to roentgen

ray at or near the height of the antibody output seems to have no effect on the height and course of the curve. It is noteworthy that in many of the animals exposed to the ray for 15-20 minutes after the introduction of the antigen, the general health did not suffer any marked disturbances, and there developed, as shown in Table 3, only a moderate degree of leukopenia. In one dog, there developed, however, a marked leukopenia — 900 on the 6th day after exposure to the ray, 650 on the 14th, 800 on the 21st, 500 on the 27th. It died on the 28th day after the exposure; there was marked anemia and emaciation; the thymus was represented by a few pinkish spots, the spleen greatly shrunken, the marrow poor in cells, and the stomach and intestine thin

TABLE 3

Effect of Roentgen Ray at Height of Antibody-Production in Dogs

Days After Injection of Antigen	Injected with Rat Blood		Injected with Goat Blood, Dog 3	Remarks
	Dog 1	Dog 2		
4	192	384	3072	
5	384	384	6144	
6	1536	1536	12288	
7	1536	1536	12288	Roentgen Ray for 20 minutes
8	1536	1536	12288	
9	3072	1536	12288	Leukocytes: Dog 1-10000, 2-13400, 3-6800
10	1536	3072	6144	
11	1536	1536	6144	Leukocytes: Dog 1-9200, 2-8000, 3-7800
12	3072	3072	3072	Leukocytes: Dog 1-4800, 2-6000, 3-5400
13	3072	3072	6144	Leukocytes: Dog 1-7000, 2-3000, 3-6800
14	1536	1536	3072	Leukocytes: Dog 1-6200, 2-4000, 3-5000
15	1536	1536	1536	
16	1536	768	768	Leukocytes: Dog 1-5100, 2-2500, 3-5200
18	1536	1536	768	Leukocytes: Dog 1-4800, 2-3600, 3-6800
22	384	384	768	Leukocytes: Dog 1-10600, 2-6200, 3-7200
30	768	384	768	
38	384	96	768	
42	192	192	96	

and distended. But there was no reduction in the amount of lysin for goat corpuscles or irregularities in its course as compared with the amount and course in the control animal.

It would require many more experiments than I have made to determine conclusively whether animals in the stage of active antibody — production, as a rule, have an increased resistance to roentgen-ray effects. The results at hand indicate that such may be the case.

It is of much interest because it bears directly on this problem that benzene, which in many ways acts like the roentgen ray, appears to have no leukopenic effect in rabbits when given in the period of anti-body-production in quantities otherwise usually effective.[3]

[2] Jour. Infect. Dis., 1916, 19, p. 69; p. 737.

In rabbits, too, roentgen ray about the 15th day after the injection of the antigen has not had in my experiments so far any definite effect on the course of the specific antibodies.

In the dog, the failure of the roentgen ray to restrain the production of antibodies after it is under way might be explained as due to the lapse of time before the radiation becomes effective, the elaboration of antibodies in the meantime passing the acme and entering on a natural decline; but it may be assumed, with at least equal reason, that the failure depends on an actual increase in the resistance to the usual roentgen-ray effects. In the rabbit, the period of active antibody-production appears much longer than in the dog, at least under the circumstances of these experiments, and consequently the first explanation suggested of the failure of the roentgen ray does not seem applicable at all to the failure of benzene to depress the antibody output when given some time after the antigen. Assuming that there develops an increased resistance to benzene and to roentgen ray, as the production of antibodies proceeds, the question arises, what is the nature of this resistance? We might adopt the hypothesis that the biologic effects of these agents are due to their influence on enzymes, as asserted by Richards[4] for radioactivity; and that, in the course of active antibody-production, special enzymes accumulate to such extent that the roentgen ray or benzene does not affect them appreciably. Whether it proves possible to furnish evidence from experiment in support of some such hypothesis as this remains to be seen.

Here I would point out that the interference of the roentgen ray under certain circumstances with the elaboration of antibodies may be a factor in the rapid development of tuberculosis in roentgenized guinea-pigs. Recently Morton[5] pointed out that this effect of the ray may be of practical value in hastening the diagnosis by guinea-pig inoculation in renal tuberculosis.

The failure of the ray to reduce the antibody-content of the blood after it has reached a certain concentration recalls the experiments of Kessel and Sittenfield[5] on radiation in the later stages of inoculation tuberculosis in guinea-pigs. The results appear to indicate that in this case radiation tends to prolong life and to promote healing of lesions. It seems then as if the effects of the roentgen ray is different not only

[4] Science, 1915, 42, p. 287.
[5] Jour. Exper. Med., 1916, 24, p. 419. See also Murphy and Taylor: Ibid., 25, p. 609.
[6] Proc. N. Y. Path. Soc., 1914, 14, p. 190.

in the early and the later phases of antibody production, but also in the early and later stages of experimental tuberculosis in guinea-pigs. This is a suggestive parallelism that merits further study.

SUMMARY

Exposure of dogs and rabbits to the roentgen ray at about the same time as antigen is introduced may restrain in high degree, and under some conditions completely, the production of antibodies as measured by the antibody content of the serum. The results correspond fully to those previously obtained from experiments on the white rat.

When antibody-production is at or near its height, exposure to the roentgen ray appears to have but little if any effect on the antibodies in the blood, and at this time dogs appear to have an increased resistance to the effects of the ray just as rabbits in the period of active production of antibodies have an increased resistance to effects of benzene. Whether there is any relationship between this apparent resistance to the effects of roentgen rays and the reported beneficial effect of the rays in the later stages of tuberculosis in guinea-pigs remains to be determined.

As stated previously[1] the results obtained from these observations harmonize with the view that antibodies are produced in the spleen, lymphatic tissues, and marrow, as these structure suffer most directly from the action of the roentgen ray; they indicate also that one reason why the lymphocyte appears to be an important agent of defense in tuberculosis and other conditions may be its power to form antibodies and that the rapid development of tuberculosis in roentgen-rayed guinea-pigs may depend, at least in part, on interference with antibody-production.

EXPERIMENTAL TRYPANOSOMIASIS: T. EQUIPERDUM INFECTION IN THE DOG

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In the previous studies from this department on the relation of the spleen to blood destruction and regeneration, we have used sodium oleate, toluylendiamin and hemolytic immune serum as hemolytic agents. Such agents, even when given in repeated doses, produced only a temporary transient effect, and it seemed desirable, in connection with the problem of iron metabolism and of the elimination of urobilin, to use a hemolytic agent which will cause a more chronic anemia. To this end, we have employed Trypanosoma equiperdum, which causes the disease dourine in horses, and is transmissible to lower animals. The disease which T. equiperdum produces in dogs presents so many points of interest that it seems wise to report the general picture of this experimental disease at this time. The chemical studies will appear in a later communication.[1]

The best discussion of the general literature of trypanosomiasis is still found in the book of Laveran and Mesnil.[2] The various blood changes caused by trypanosomes are reported in the recent works of Jakimoff and Wassilewsky,[3] Lafranchi,[4] and Boycott and Price-Jones.[5]

Although many experimental studies of the changes brought about by various strains of trypanosomes are found in the literature, few if any studies have been made with T. equiperdum. In a study of nagana, or tse-tse fly disease (caused by T. brucei), Kanthack, Durham, and Blandford[6] found changes similar to those produced by us in the various animals studied. These observers found that the duration of the disease depended largely on individual susceptibility. Rats survived from 6 to 26 days, mice from 8 to 25, rabbits 13 to 58, dogs 14 to 26, and guinea-pigs from 20 to 183 days. The disease could be transmitted by inoculation, subcutaneously, intravenously, intraperitoneally, in the anterior chamber of the eye, or in skin scratches. Trypanosomes were recovered from the blood, spleen, bone marrow, lymph nodes, aqueous humor, testicular juice, and all serous and edematous fluids. The salient symptoms were wasting, loss of power, fever, edema, eye changes, anemia, and urobilinuria. As in our series, it was noticed that wounds did not heal well and frequently became septic.

[1] Dubin, H., and Pearce, R. M.: The Elimination of Iron and Its Storage in the Liver and Spleen in Experimental Anemia, II, Jour. Exp. Med., in press.

[2] Paris, Masson & Cie, Ed. 2, 1912.

[3] Compt. rend. Soc. de biol., 1910, 128, p. 309.

[4] Fol. Hemat., 1912, Arch. 13, p. 55.

[5] Jour. Path. and Bact., 1913, 17, p. 347.

[6] Proc. Roy. Soc., 1899, 64, p. 100.

Moore and Breinl[7] have studied the life history of T. equiperdum in rats and explain its method of fission, its metamorphoses, and its three maximum phases.

Methods

Dogs alone were used in our experimental work. At first, dogs were injected intraperitoneally with infected rat's blood containing approximately 600,000,000 T. eqiperdum. As, however, infection did not always result, all subsequent inoculations were given intravenously and the procedure adopted of injecting 1 c.c. per kilo of body weight, of whole blood from a heavily infected dog, without further attention to the actual number of trypanosomes present.

Such inoculations were successful in all instances except two: in one of these a second attempt proved successful, in the other, no further attempt was made. A few attempts to transmit the infection to guinea-pigs and rabbits were entirely unsuccessful. Our conclusions are based on the study of 28 infected dogs.

Clinical Course of the Disease

Incubation Period.—The incubation period varied from 3 to 8 days, and directly with the size of the dose. With successive transmission through dogs, the virulence of the infection was increased, so that both the incubation period and the duration of the disease was shortened, and the maximum anemia more quickly reached.

General Symptoms.—Throughout the incubation period, up to the appearance of trypanosomes in the circulating blood, the dogs appeared normal. With the appearance and multiplication of trypanosomes in the blood, the dogs began to show a marked general weakness, with considerable loss of weight, but it was also noticed that a severe anemia and even a fatal termination might be reached without such symptoms being extreme. Lethargy and tendency to sleep were conspicuous but it was difficult to distinguish between these conditions and the associated symptoms of weakness. The effects of decubitus appeared on prominent portions of the body, and although emaciation was undoubtedly a predisposing cause, there was a marked decreased tendency toward healing both in these and in various operative wounds.

A characteristic feature of the condition, which has also been noted clinically in other forms of trypanosomiasis, was the frequent occurrence of edema. In one dog this took the form of a marked general anasarca, while in others it was limited to edema of the external genitalia and localized swellings around the tendo Achillis. Living trypanosomes were readily obtained in considerable number from the edema fluid. Yorke[8] explains that this edema as due to a dilution of the toxic blood plasma following changes in osmotic pressure rather than to the actual presence of the trypanosomes.

Another almost constant condition was the appearance of marked eye lesions —keratitis, iritis, hemorrhagic exudates in the anterior chamber—changes which have been frequently commented on by other writers on trypanosomiasis. Living trypanosomes were found in the humors of eyes presenting these lesions. The nature and pathology of these eye lesions will be considered in detail elsewhere.[9]

The urine of these dogs was concentrated, of high specific gravity and high color. Sugar was never found. Albumin was present only occasionally in

[7] Proc. Roy. Soc., 1908, 80, p. 288.
[8] Ann. Trop. Med. and Parasit., 1911, 5, p. 127.
[9] Woods, A. C., and de Schweinitz, G. E.: Arch. Ophth., 1917, 42, p. 431.

very faint traces. Casts were never found. Bile pigment (Gmelin and Rosen-
bach tests), however, was usually present, although no staining of the skin or
mucous membrane was ever noted.

In the only two animals in which death occurred in the natural course of
the infection, the mode of death was very suggestive of a cerebral lesion.
A few hours before death, the lethargy increased to a condition of stupor.
This was succeeded by unconsciousness, complicated by semipurposeful con-
vulsions in which the unconscious animal, lying on its side, performed rapid
running motions with all legs. Between convulsions dyspnea was marked and
the pulse rapid. Although this was probably due to actual cerebral involve-
ment, no thrombi, emboli, or other gross lesions could be discovered.

Almost as soon as trypanosomes are found in the blood signs of anemia
are manifest, both in the hemoglobin and red blood cell count. The hemo-
globin tends at first to fall more rapidly but the fall in red cells is mainly
parallel. In a few weeks, the hemoglobin may fall to 40 or lower, the erythro-
cytes to less than 3,000,000 per c.mm. The course and character of the anemia
in a single dog is shown in Table 1, and composite curves of changes in red
cells and hemoglobin in several dogs in Charts 1 and 2.

TABLE 1

T. Equiperdum Infection in the Dog

Infection Time in Days after Injection of 3 C.c. Whole Blood, about 150,000,000 T. equiperdum	Hemo-globin	Red Cells	Leuko-cytes	Differential Counts			
				Polynu-clears	Small Lympho-cytes	Large and Transi-tional Lympho-cytes	Eosino-phils
Before	105	6,580,000	12,400	46	40	5	9
2	98	7,020,000	19,000	66	20	10	4
8	...	6,600,000					
11	96	5,600,000	9,600	54	40	2	4
18	54	3,800,000	6,600	52	45	2	1
26	50	3,400,000	5,400	73	27	...	...
33	46	3,440,000	8,200	79	14	7	...
40	50	3,900,000	45,200	84	11	5	...
46	...	... ...	.....	..	..	...	...

* Hemolysis in various strengths of salt solution, 0.30 to 0.46%.

The leukocytes after showing a temporary leukocytosis lasting only a few
days, exhibit a progressive leukopenia during the ensuing period of the disease.
In a few cases in which counts were made shortly before death, a terminal
leukocytosis was observed (as high as 45,000), but it was not definitely ascer-
tained whether or not this was due to terminal secondary infection (Chart 3).

Differential counts show that the initial and terminal leukocytoses are due
chiefly to increase in the polymorphonuclear cells. The eosinophils tend to
disappear during the course of the infection. During the leukopenic stage, the
lymphocytes show both a relative and actual increase (Chart 4).

Nucleated red cells, both normoblasts and megaloblasts, occasionally appear
in the blood stream soon after anemia has developed and persist for 2 or
3 weeks. They nearly always disappear a few weeks before death, in spite
of the fact that the bone marrow is always found at necropsy to be hyper-
plastic. Polychromatophilia (which is occasionally found in the normal dog's
blood) is not marked; neither is there much anisocytosis or poikilocytosis.

The resistance of the erythrocytes (measured by hypotonic salt solution), though not showing very striking changes, tended to be decreased. This was especially true of the maximum resistance, during the earlier part of the infection (Chart 5).

Blood platelets, examined by the Wright and Kinnicutt method, were apparently decreased, but the counts of these were so variable that we have not much confidence in the results obtained (Table 1).

Estimation of the percentage of skeined or reticulated erythrocytes (by vital staining with brilliant cresyl blue) showed in the earlier stages of the infection an increase, indicating an attempt at bone marrow regeneration. This latter was seldom sufficient, however, to cause the appearance of normoblasts in the peripheral blood stream. Seidelin[10] also has found a reduction in platelets and an increased number of 'metachromatic erythrocytes' in trypanosome infection (Table 1).

Evidences of blood destruction, as measured by increased elimination of urobilin and increased storage of iron in liver and spleen were present. These results will be considered elsewhere.[1]

TABLE 1—*Continued*
T. EQUIPERDUM INFECTION IN THE DOG

Fragility		Skein Cells, per Cent.	Platelets	Weight of Dog, Kg.	Remarks
Complete Hemolysis*	Beginning Hemolysis*				
32	46	0	80 M.	9.7	
34	46	...	......	10.2	Trypanosomes in circulating blood
36	46				
36	46	1.5	......	....	
32	44	2	......	11.2	Keratitis
32	44	0.6	73 M.		Diarrhea
30	44	0.4	50 M.	10.3	
	...	...	.....	...	Died

THE EFFECT OF SPLENECTOMY

In the course of the studies of the iron metabolism in these animals, a number of dogs were splenectomized, some before and some after infection with trypanosomes. In both instances the animals did poorly. Six dogs splenectomized at the height of the trypanosome infection died within 2 weeks, and the majority within 1 week. These dogs showed no appreciable change in the blood picture beyond the usual leukocytosis following splenectomy. Likewise, with one exception, the 5 dogs splenectomized before infection with trypanosomes all died within 11 days after inoculation, and within 2 or 3 days following the appearance of trypanosomes in the circulating blood. The exception was a dog splenectomized on January 22. It showed the early leukocytosis and a gradually developing, moderate anemia characteristic of splenectomized dogs. It was inoculated with trypanosomes on March 31, and lived 31 days after inoculation. During this period the anemia gradually grew more intense, the hemoglobin falling to 35% and the red blood cells to 3,940,000, on the day of death.

10 Jour. Path. and Bact., 1914-1915, 19, p 315.
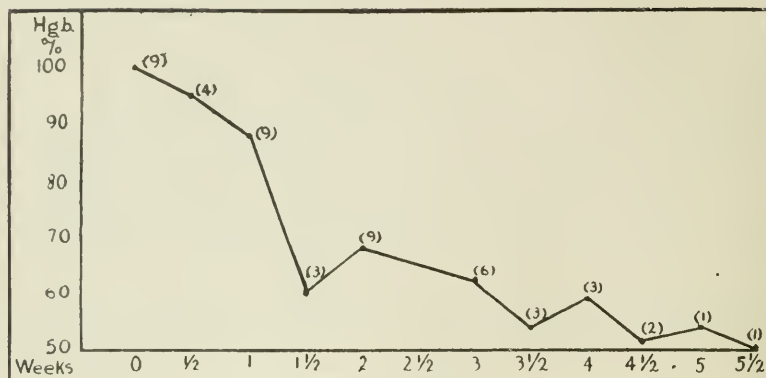
Chart 1.—Hemoglobin counts after trypanosome infection. Composite chart. Figures in this and subsequent charts indicate the numbers of animals examined.
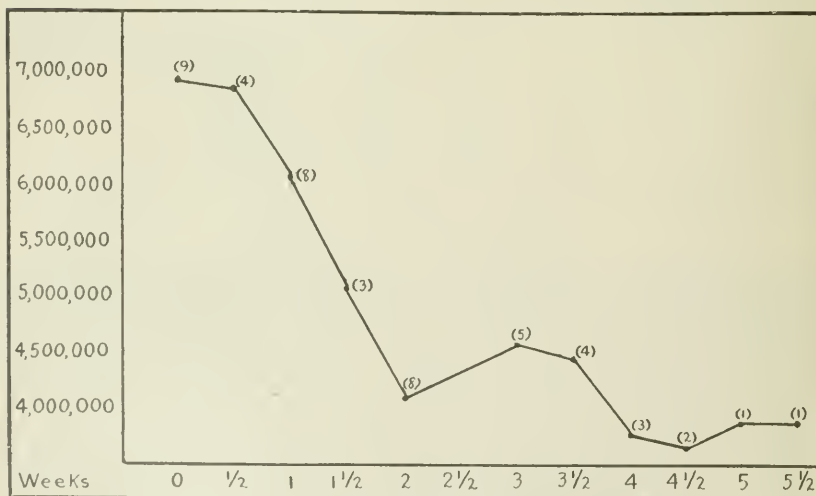
Chart 2.—Red blood counts after trypanosome infection. Composite chart.
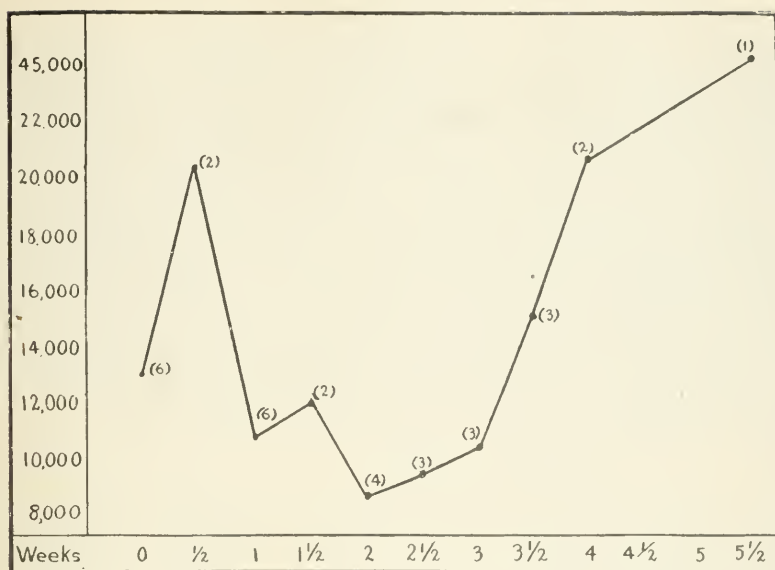
Chart 3.—Leukocyte counts after trypanosome infection. Composite chart.
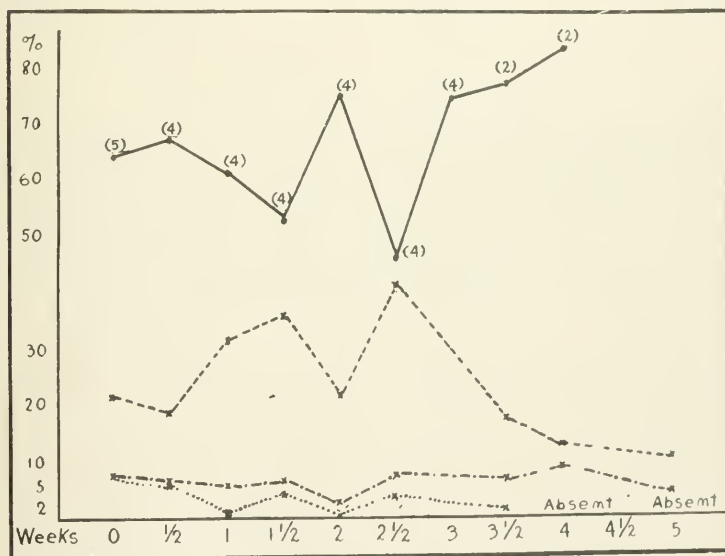
Chart 4.—Differential counts after trypanosome infection. Composite chart. Poly-
morphonuclear leukocytes expressed with straight line ————; small lymphocytes expressed
with dashed line — — — —; large and transitional forms expressed with broken line
— . — . — . — . ; eosinophils expressed with dotted line ....

The Effect of Salvarsan on the Disease

Riquer[11] in Germany, and Schamberg, Kolmer, and Raiziss,[12] in this country, have shown that salvarsan, and its American counterpart, arsenobenzol, have a chemotherapeutic effect on trypanosomiasis. Given intravenously in the dosage of 0.01 gm. per kilo of bodyweight, the blood of dogs infected with T. equiperdum becomes quickly sterile often after one injection. To insure a permanent cure, we have found it necessary to give 3 injections on successive days, and to follow this with 3 or more injections at subsequent 3-day intervals.

Following the intravenous administration of arsenobenzol, all clinical manifestations and symptoms disappeared in a most prompt and striking manner. Within 3 days after the first injection the lethargy and weakness disappeared, the edema subsided, the decubitus lesions began to heal rapidly, and the ocular symptoms cleared up. After 10 days of treatment the dogs appeared normal.

The blood pictures in the dogs, in which a permanent cure was effected, have not been followed. Table 2 shows the prompt regeneration in the blood following 3 injections of arsenobenzol. The trypanosome infection later recurred in this dog with the development of a progressive anemia.

TABLE 2

Regeneration of the Blood After Arsenobenzol

	Hb %	Red Cells	Leukocytes
February 28. Before treatment............. .... 0.01 gm. arsenobenzol per kilo of body weight on March 1, 2 and 3	32	2,700,000	4,500
March 3	63	4,290,000	19,000
March 10	58	5,310,000	20,300
March 17	78	5,270,000	12,000

Pathologic Anatomy

On postmortem examination the most important changes found were: (1) degenerative changes in the parenchymatous organs; (2) hyperplasia of the marrow and the long bones; and (3) enlargement of the spleen. The 1st and 2nd of these do not demand detailed discussion, as they are the usual concomitants of experimental anemia. The splenomegaly, however, is worthy of special comment. In the normal dog the weight of the spleen varies greatly with an average of about 25 gm. In a special series we have found the extremes to be 11 and 55 gm. respectively, the majority, however, falling between 17 and 26. The size of the normal spleen varies likewise; its length being 11-19 cm., its greatest widths 3-6 cm., and thickness 0.6-1.8 cm. In animals infected with trypanosomes we have found the weight to be greatly increased. In 8 animals it ranged from 110 to 150 gm. and in 5 from 50 to 87 gm. The increase in size was not in proportion to the weight, the largest spleen (150 gm in weight) measuring 26 cm. in length, 7.5 cm. in greatest width and 4.5 cm. in thickness. In its gross appearance the spleen is purplish-red, with a smooth distended capsule and on section soft, succulent, and of bright red color, with very prominent malpighian bodies. Microscopically, congestion is the chief characteristic but there is also an increase of endothelial cells and of old blood pigment. Many of the endothelial cells are phagocytic for erythrocytes and the malpighian bodies appear unusually cellular.

[11] Ztschr. f. Immunitätsforsch. u. exper. Therap., 1913, O., 21, p. 92.
[12] Jour. Am. Med. Assn., 1915, 65, p. 2142.

SUMMARY

Dogs may be readily infected with T. equiperdum and a severe anemia be produced. The incubation period varies from 3 to 8 days, and a fatal termination results in from 3 to 7 weeks. By successive transmission through dogs the virulence of the infection may be increased so that both incubation period and duration of the disease may be shortened and the maximum anemia more quickly reached.

With the appearance of trypanosomes in the circulating blood, the animals show general weakness, loss of weight, lethargy, and a lessened
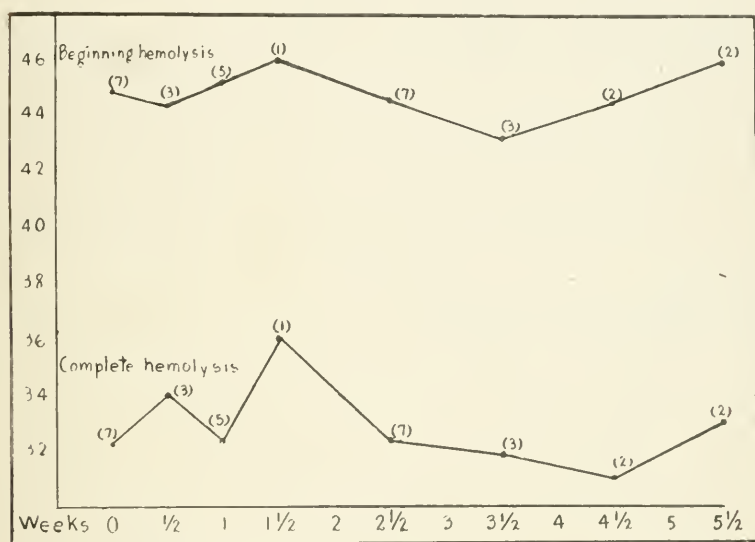
Chart 5.—Resistance tests after trypanosome infection. Composite chart. Of two animals examined 5½ weeks after infection, one showed a slight increase of both maximal and minimal resistances, the other a decrease of both resistances, so that it seems better not to include these figures.

tendency to the healing of wounds. Subcutaneous edema is a common manifestation and may appear as a general anasarca or be limited to the genitalia or the extremities. The edema fluid contains living trypanosomes. Another interesting and almost constant lesion is keratitis. Choluria is constantly present without evidence of jaundice in the skin or mucous membrane.

The anemia which develops is progressive and of the hemolytic type. The hemoglobin may fall to 40, and the red cells to less than 3,000,000 per c.c.

Attempts at regeneration are shown by the increased number of skeined cells, occasional nucleated reds in the peripheral stream, and hyperplastic bone marrow at necropsy. That this attempt at repair is entirely inadequate, however, is shown not only by the progressive lowering of hemoglobin and erythrocyte count, but also by the leuko-penia, diminution in platelets, later lowering of percentage of skeined cells, and disappearance of nucleated red blood cells from the peri-pheral blood stream.

The principal pathologic changes are the usual degenerative lesions of anemia in the parenchymatous organs, hyperplasia of the bone marrow and a great enlargement of the spleen.

Splenectomy at the height of the trypanosome infection has no beneficial influence, but rather the reverse, on the anemia or the course of the infection. Animals splenectomized before infection died more quickly than did those with intact spleen; as a rule, within 2 or 3 days after the appearance of trypanosomes in the circulating blood.

The intravenous injection of arsenobenzol, 3 injections at intervals of 3 days, has been followed by a disappearance of all symptoms and of trypanosomes from the blood and of a prompt improvement in the blood picture. In some instances, however, the disease has recurred on the discontinuance of treatment.

COMPLEMENT FIXATION IN EXPERIMENTAL TRYPANOSOMIASIS

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It has long been known that the serum of animals infected with trypanosomes will give complement binding with antigens prepared from trypanosomes or the extracts of organs of animals dead from trypanosomiasis. As a part of a general study of experimental trypanosomiasis, we have examined the serum of a number of infected dogs by the complement binding reaction, with the idea of determining: (1) the time of occurrence of the reaction; (2) the relationship of the reaction to the various symptoms and pathologic changes; (3) the specificity of the reaction; (4) its relationship to the Wassermann reaction, and (5) the effect of salvarsan on the reaction.

HISTORICAL

The literature on this subject was abstracted in 1913 by Mohler, Eichorn, and Buck[1] who gave much attention. to the preparation of a suitable antigen. They found that extracts of organs of horses dead of dourine did not yield satisfactory antigens but that emulsions of trypanosomes, obtained by laking the blood of rats infected with trypanosomes, yielded a satisfactory antigen. The difficult method of obtaining this antigen (centrifuging, pipetting of the trypanosomes and repeatedly washing) led these observers to prepare a salt solution extract of the spleen of rats just dead of surra. This antigen was generally satisfactory. It was found necessary to prepare this antigen fresh about every 10 days, and to titrate on the day of the test against known posititive and known negative sera, in order to guard against the occasional nonspecific fixation and anticomplementary action of such an antigen. Using these precautions the authors uniformly obtained positive results with positive sera, and negative results with negative sera.

Watson,[2] in 1915, reports successful results by the use of an emulsion of trypanosomes as antigen. He likewise states that the extract of the spleen of rats dead of surra yields a satisfactory, but not so sensitive antigen.

PREPARATION OF ANTIGEN

Antigens were prepared by grinding in 30 c.c. of normal salt solution, the spleen of a rat, either heavily infected with T. equiperdum or just dead from such infection. This emulsion was filtered and titrated against two known negative sera, and used in one third the anticomplementary dose, provided the

[1] Jour. Agric. Res., 1913-1914, 1, p. 99
[2] Parasitol., Cambridge, 1915, 8, p. 156.

anticomplementary dose was not over $1:6$. If the antigen was anticomplementary, or gave nonspecific fixation with the known negative sera, a phenomea occasionally observed by us and by the previous workers in this field, this antigen was discarded and a fresh one prepared. A fresh antigen was always prepared and titrated on the day of the test. Old antigens were never used. In several of the reactions an antigen composed of an emulsion of T. equiperdum, prepared according to the technic given by Mohler, Eichorn and Buck, was used. This antigen did not appear to be in any way superior to that composed of the spleen extract.

To control the specificity of the reaction, the various sera were all examined against an antigen prepared from the spleen of a normal rat. Wassermann reactions, against crude alcoholic extract of human heart as antigen, were made with all sera.

TABLE 1

COMPLEMENT FIXATION WITH TRYPANOSOME ANTIGEN AND WITH SPLEEN OF NORMAL RAT

Animal	Antigens	April 20	April 21	April 24	April 28
17-64 Splenectomized. Trypanosomes appeared in blood on April 28	Trypanosome spleen	Neg.	Infected	Neg.	++
	Normal spleen	Neg.		Neg.	Neg.
17-65 Splenectomized. Trypanosomes appeared in blood on April 24	Trypanosome spleen	Neg.	Infected	Neg.	++
	Normal spleen	Neg.		Neg.	Neg.
17-66 Trypanosomes appeared in blood on April 24	Trypanosome spleen	Neg.	Infected	Neg.	+++
	Normal spleen	Neg.		Neg.	Neg.
17-67 Trypanosomes appeared in blood on May 2	Trypanosome spleen	Neg	Infected	Neg.	++
	Normal spleen	Neg.		Neg.	Neg.
17-69 Trypanosomes appeared in blood on April 24	Trypanosome spleen	Neg.	Infected	Neg.	+
	Normal spleen	Neg.		Neg.	Neg.
17-72 Trypanosomes appeared in blood on April 24	Trypanosome spleen	Neg.	Infected	Neg.	++
	Normal spleen	Neg.		Neg.	Neg.
17-73 Trypanosomes appeared in blood on April 24	Trypanosome spleen	Neg.	Infected	Neg.	+
	Normal spleen	Neg.		Neg.	Neg.

TECHNIC OF COMPLEMENT FIXATION

The technic used was essentially that described by Snow and Cooper,[3] and outlined in other papers appearing from this laboratory. Any natural anti-sheep hemolysin in the serum to be examined was absorbed by adding 4 c.c. of a 5% suspension of sheep erythrocytes to 1 c.c. of the serum, later removing the cells by centrifuging and using the 20% serum in the test. Three test tubes, containing respectively 0.2, 0.5, and 1.0 c.c. of the dilute serum, together with the usual serum, hemolytic and antigen controls, were run in every instance. A +++ reaction indicates complete or practically complete inhibition of hemolysis with all quantities of serum, a ++ reaction similar inhibition with the 2 larger quantities of serum, and a + reaction such inhibition only in the tube containing 1.0 c.c. of serum.

Sensitized cells were used in all tests. Complement was titrated in the presence of antigen on the morning of the test.

[3] Am. Jour. Med. Sc., 1916. 152, p. 185.

SELECTION OF ANIMALS

The serum of a number of normal dogs were examined against these several antigens—extract of trypanosome spleen, extract of normal spleen, and the Wassermann antigen—in a preliminary reaction. Seven dogs whose sera were not anticomplementary and gave negative reactions with these antigens, were selected for use in this work. Two of these dogs were splenectomized before infection. All dogs were injected with 10 c.c., per kilo of bodyweight, of the blood of a dog heavily infected with trypanosomes.

RESULTS

One very constant finding was noted with respect to the dogs, which from a review of the literature, does not seem to have been observed in the case of

TABLE 1—*Continued*

COMPLEMENT FIXATION WITH TRYPANOSOME ANTIGEN AND WITH SPLEEN OF NORMAL RAT

May 2	May 5	May 9	May 12	May 16	Remarks
+	+	Died May 8			
Neg.	Neg.				
Died May 2					
+++	++	Serum anticomplementary	Serum anticomplementary	Serum anticomplementary	Treatment with arseno-benzol begun on May 16. See Table 3
Neg.	Neg.				
+++	++	Serum anticomplementary	Serum anticomplementary	Serum anticomplementary	Died May 22
Neg.	Neg.				
++	+	Serum becoming anticomplementary	Serum anticomplementary	Splenectomy in May 15	
Neg.	+				
++	++	Serum becoming anticomplementary	Serum anticomplementary	Serum anticomplementary	Splenectomized May 22
Neg.	·+				
++	+++	++	+++	Serum anticomplementary	Treatment with arseno-benzol begun on May 16. See Table 3
Neg.	Neg.	Neg.	Neg.		

other animals. As the trypanosomes multiplied in the blood of the dogs, and symptoms of trypanosomiasis developed, the sera of the dogs became anticomplementary. This point will be discussed later.

The results of the fixation reactions with trypanosome antigens and antigens of the spleen of a normal rat, are shown in Table 1.

The fixation reaction first appears about 8 days after infection. In all except 1 instance, trypanosomes appeared in the circulating blood before the complement fixation became positive. One dog, on the other hand, gave a positive reaction 4 days before the appearance of trypanosomes in the blood stream, an occurrence in accord with the findings of other observers. .

The fixation appeared to be specific. Five dogs gave persistently negative reactions with the extract of a normal rat's spleen, while a positive reaction was obtained with antigens of the spleen of a rat infected with trypanosomes. Two dogs, 17-69 and 17-72, gave a weak reaction with the normal spleen antigen on 1 day, May 2. Three days later both these sera were partially anticomplementary and it seems probable that the weak reaction with the normal spleen antigen may be in some way connected with this phenomena.

RELATIONSHIP WITH THE WASSERMANN REACTION

Owing to the great tendency of dog's sera to give nonspecific fixation with cholesterin antigens, simple alcoholic extract of human heart, of a titer of 1 : 10, was used in the Wassermann reaction. Table 2 shows the Wassermann reaction of the different sera at different periods after infection. A variable and inconstant fixation of complement with the Wassermann antigen was shown by these sera.

EFFECT OF SALVARSAN ON COMPLEMENT FIXATION

It has been shown by Riquier,[4] and by Schamberg, Kolmer and Raiziss[5] that salvarsan, and its American reproduction arsenobenzol, have a chemotherapeutic effect in trypanosomiasis. After 1 or more injections of arsenobenzol, given intravenously in the dosage of 0.01 gm. per kilo of bodyweight, trypanosomes disappear from the circulating blood of infected animals, there is a prompt clearing up of all symptoms, and the animals return to normal, provided sufficient injections of arsenobenzol are given.

Two dogs have been treated with arsenobenzol in order to determine the effect on the complement fixation reaction. (The arsenobenzol was furnished by Dr. J. A. Kolmer, to whom we express our thanks.) The sera of both these dogs had given strongly positive reactions, and had become anticomplementary prior to the first injection of arsenobenzol. Injections were given on 3 consecutive days, and on every 3rd day thereafter. Sera was collected on the 6th and 9th days after the first injection. These sera, however, were still anticomplementary. One of these dogs died a week later. The remaining dog remained in good condition and the serum was again taken, 21 days after the date of the first injection. This serum was not anticomplementary, and gave completely negative reactions with both the trypanosome and the Wassermann antigens. This result is shown in Table 3. In this one instance, the anti-complementary action and the complement fixation properties with trypanosome and Wassermann antigens were dissipated by treatment with arsenobenzol.

THE ANTICOMPLEMENTARY EFFECT OF TRYPANOSOMES

The anticomplementary phenomena shown by the serum of these dogs at the height of the infection with trypanosomes has already been commented on. This phenomenon disappears following the sterilization of the blood with arseno-benzol. It has seemed possible to us that this complementary action of the serum might be due either to the presence of the large number of trypanosomes in the blood, or to metabolic products freed by the trypanosomes in the blood.

In the hope of casting some light on this problem, we have prepared an emulsion of washed trypanosomes after the method given by the previous workers already mentioned. This emulsion was added in the proportion of 1 : 10 and 1 : 20 to the fresh serum of a normal dog. These dilutions were incubated at 38 C. for varying periods, the trypanosomes removed by centrifuging and the serum then tested for anticomplementary action. Table 4 illustrates the results of this experiment.

[4] Ztschr. f. Immunitätsf. u. exper. Therap., 1913, O., 21, p. 92.
[5] Jour. Am. Med. Assn., 1915, 65, p. 2142.

TABLE 2

Wassermann Reactions

Animal	April 24	April 28	May 2	May 5	May 9	May 12	May 16	June 5
17-64	Neg.	Neg.	Neg.	Neg.	Dead			
17-65	Neg.	+	Dead					
17-66	Neg.	++	++	+	Serum anticomplementary			Neg. (See Table 3)
17-67	Neg.	++	+++	++	Serum anticomplementary			Dead
17-69	Neg.	Neg.	Neg.	+	Serum anticomplementary			Dead
17-72	Neg.	Neg.	+	+	Serum anticomplementary			Dead
17-73	Neg.	Neg.	Neg.	+	Neg.	Serum anticomplementary		Dead

TABLE 3

Effect of Salvarsan on Complement Fixation

Animal	Antigen	May 5	May 12	May 16		May 21	May 24	June 5
17-66	Trypanosome spleen	++	Serum anticomplementary		0.01 gms. arseno-benzol on May 16, 17, 18, 21, and 24	Serum anticomplementary		Neg.
	Wassermann	+	Serum anticomplementary			Serum anticomplementary		Neg.
17-73	Trypanosome spleen	+++	+++	Serum anticomplementary		Serum anticomplementary		Died on June 2
	Wassermann	+	Neg.	Serum anticomplementary		Serum anticomplementary		~

TABLE 4

Anticomplementary Action of Trypanosomes

Dilution of Trypanosomes with Fresh Serum								With Salt Solution	
1:10, 2 Hrs. at 38 C., 14 Hrs. in Ice-box	1:10, 4 Hrs. at 38 C., 12 Hrs. in Ice-box	1:10, 16 Hrs. at 38 C.	1:20, 2 Hrs. at 38 C., 14 Hrs. in Ice-box	1:20, 4 Hrs. at 38 C., 12 Hrs. in Ice-box	1:20, 16 Hrs. at 38 C.	Normal Serum 16 Hrs. at 38 C.	Normal Serum 16 Hrs. in Ice-box	1:10, 16 Hrs. at 38 C.	1:20, 16 Hrs. at 38 C.
∓	∓	+	−	−	+	∓	−	+	+

— indicates complete hemolysis, ∓ moderate inhibition of hemolysis, and + complete inhibition of hemolysis.

Trypanosomes in the concentration of 1:10 in fresh serum incubated at 38 C. for 2 and 4 hours, produced a moderate anticomplementary action. In the concentration of 1:20, incubated at 2 and 4 hours, no anticomplementary action was produced. In both the concentration of 1:10 and 1:20 incubated for 16 hours, the trypanosomes rendered the serum completely anticomplementary. A similar effect was rendered in normal salt solution. Straight serum incubated at 38 C. for 16 hours was only slightly anticomplementary, while the same serum kept in the ice-box showed no anticomplementary action.

It seems probable, therefore, that the anticomplementary action developed by the serums of dogs as a result of infection with the trypanosome equiperdum is due to metabolic products liberated by the trypanosomes into the blood.

SUMMARY

Dogs infected with T. equiperdum develop complement fixation with a specific antigen within 8 days after inoculation. An easily prepared and a very satisfactory antigen is the salt solution extract of the spleen of a rat heavily infected with trypanosomes or dead from the infection. The complement fixation usually follows the appearance of trypanosomes in the blood, although it may occasionally precede the appearance of trypanosomes. The complement fixation, however, always antedates the appearance of symptoms.

Dogs infected with trypanosomes frequently give a positive Wassermann reaction.

Within 3 weeks after the appearance of trypanosomes in the blood, the serum of the infected dog becomes strongly anticomplementary. This anticomplementary phenomenon appears to be due to the liberation of anticomplementary substances into the blood by the invading trypanosomes.

The blood is rendered sterile, and all clinical symptoms clear up following the intravenous injection of arsenobenzol, and in the only complete experiment at hand, the anticomplementary action and complement fixation properties with the trypanosome and Wassermann antigens likewise disappeared.

OBSERVATIONS ON THE STABILITY OF THE ERYTHROCYTES OF THE OX, PIG, AND SHEEP

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WASHINGTON, D. C.

In doing a parallel series of Wassermann reactions by the Noguchi method with acetone insoluble extract of heart as antigen, and by the drop modification of the original Wassermann with alcoholic extract of heart as antigen, difficulty was experienced in obtaining sheep blood owing to the fact that no sheep were being killed at the local abattoir. Ox blood was readily obtainable and it was decided to make an anti-ox erythrocyte amboceptor. A working amboceptor was made after 5 intravenous injections into a rabbit. Several others have been made since, but never with less than 5 intravenous injections. The ox cell system worked so well and was in such close agreement with the Noguchi method that the latter was gradually abandoned and the ox system used exclusively, employing 2 antigens, alcoholic extract of human heart and cholesterinized extract.

After reading the account by Kolmer and Casselman[1] on natural hemolysins in human serum and Gradwohl's account[2] of his modification of the Bauer and Hecht-Weinberg method of performing the Wassermann reaction, I became curious to try out a series of human sera on the corpuscles of the 3 animals commonly found in abattoirs.

For 10 weeks the corpuscles of as many different oxen, pigs, and sheep were tested with a varying number of human sera, 123 in all. The various bloods were collected in sterile citrate solution once a week at the abattoir. As all 3 animals were not always killed on the day before doing the Wassermann tests, the bloods were often kept on ice at the abattoir or in the laboratory for 1 to 3 days before they were used. The weakening effect on the corpuscles by keeping them 3 days is shown in Table 2, Animals 4. The effect is insignificant, but is least marked on the ox corpuscles. Following the regular Wassermann tests 3 series of tubes were set up and to each tube was added 0.5 c.c. of salt solution, 1 drop of fresh guinea-pig serum, 1 drop of the various sera to be tested, and 0.5 c.c. of a 3% suspension of the 3 kinds of cells being tested. The drops of sera were delivered with a capillary pipet of 1 mm. outside diameter. The guinea-pig serum was known not to be hemolytic for the various cells being tested and as the human sera had all been previously used in the regular test they were known not to be anticomplementary. The 3 series of tubes were done in duplicate, 1 set for the unheated

[1] Jour. Infect. Dis., 1915, 16, pp. 441-447.
[2] Jour. Amer. Med. Assn., 1917, 68, p. 514.

active sera and the other set for those portions of the sera which had been
inactivated at 56 C. for use in the regular Wassermann tests. The tubes were
incubated in a water bath for 1 hour at 37 C., then placed in the ice box
for 2 or more hours for the corpuscles to settle out when the results were
read. The degrees of hemolysis were estimated by the eye. Five per cent. is
a mere trace and is negligible and was only used to distinguish tubes in which
the supernatant fluid was absolutely white. Ten per cent. is a pronounced
trace, 25%, 50%, 75%, and 100% represent increasing grades of hemolysis.
These degrees of hemolysis correspond with the following Wassermann read-
ings: 0% and 5%, + + + +; 10% and 25%, + + +; 50%, + +; 75%, +;
100%, negative, provided that the readings had been made from the use of
only 1 tube and 1 volume of serum. In actually performing the test the
degrees of hemolysis are determined from the use of 2 tubes, 1 containing
a single and the other a double quantity of the serum to be tested. Table 1
gives a summary of the results.

TABLE 1

PERCENTAGES OF HUMAN SERUM CAUSING INCREASING GRADES OF HEMOLYSIS WHEN ACTING
ON THE ERYTHROCYTES OF THE OX, PIG, AND SHEEP IN THE PRESENCE
OF FRESH GUINEA-PIG SERUM

Extent of Hemolysis	Active Human Serum			Inactivated Human Serum		
	Ox	Pig	Sheep	Ox	Pig	Sheep
0%	40	47	0	69	78	3
5%	13	15	0	15	6	1.6
0 and 5% together	53	62	0	84	84	4.6
10%	15	16	1.6	11	5.7	3
25%	14	8	5.7	3	5.7	7
50%	8	5	7	2	3	26
75%	7	6	18.7	0	0.8	17
100%	3	3	67	0	0.8	42

This table shows that all the corpuscles are more resistant to the inactivated
sera than they are to the active sera, a result naturally to be expected, although
in many instances there are no differences or no appreciable differences. The
pig corpuscles are relatively more resistant to the active sera than are the
ox corpuscles, but not so far as the inactivated sera are concerned. The active
sera produced essentially no hemolysis of the ox cells in 53% of the cases;
of the pig cells in 62%, while all of the sheep cells showed some grade of
hemolysis with active sera. With the sera inactivated the percentages are for
the ox cells, essentially no hemolysis, 84%; with the pig cells, 84%; and with
the sheep cells, 4.6%. Complete hemolysis by the active sera on the ox cells
occurred in 3% of the cases; on the pig cells in 3%; and on the sheep cells,
67%. When the inactivated sera were used the figures are 0% for the ox
cells, 0.8% for the pig cells, and 42% for the sheep cells.

In no instance were the sheep cells more resistant than those of the ox or
pig. In a very few instances, sera numbers 12 inactivated, 17 inactivated, 20
active, 31 active, 101 active, 104 active, 111 inactivated, 113 inactivated, the
cells of all 3 animals showed the same degree of resistance. The pig and ox
cells parallel one another very closely. Any difference in resistance between
pig and ox is usually in favor of the pig. In a few instances, the ox cells
were more resistant than those of the pig, sera numbers 29, 35, 54, 58, 61, 62,
64, 65, 66, 69, 71, 119, active; and 20, 22, 29, 31, 32, 35, 47, 62, 64, 65, 66, 68,

71, 119, 120, 122, inactivated, and in these cases the difference in degree of hemolysis was usually not marked.

No satisfactory explanation of these facts can be offered. To say that human serum contains a natural antisheep erythrocyte amboceptor is simply another way of expressing the observed facts. Zoologically, sheep and oxen are closely allied and one would expect their corpuscles to behave similarly under the influence of a serum obtained from as zoologically remote an animal

TABLE 2

Degree of Hemolisis of the Erythrocytes of the Ox, Pig and Sheep, Expressed in Estimated Percentages, when Placed in Hypotonic Salt Solutions. No. 4a Represents the Same Corpuscles as those from Animal 4, but they Were Tested Three Days Later after Having Been Kept on Ice

Hypotonic Salt Solutions	Ox Number						Pig Number						Sheep Number					
	1	2	3	4	4a	5	1	2	3	4	4a	5	1	2	3	4	4a	5
0.75%	0	0	0	0	0	0	0	5	5	0	0	0	5	0	0	0	0	0
0.70%	0	0	0	0	0	0	5	5	10	0	5	0	10	0	5	0	0	0
0.65%	0	0	0	0	0	0	10	10	25	5	10	0	50	5	10	0	5	0
0.60%	5	0	0	5	5	0	25	25	25	5	25	10	75	10	25	5	10	0
0.55%	10	5	0	10	10	0	50	50	50	25	75	50	100	25	50	25	75	0
0.50%	75	25	5	10	25	0	75	75	75	50	100	50	100	75	75	100	100	5
0.45%	100	75	10	75	75	5	100	100	100	100	100	100	100	100	100	100	100	50
0.40%	100	100	50	100	100	10	100	100	100	100	100	100	100	100	100	100	100	75
0.35%	100	100	100	100	100	50	100	100	100	100	100	100	100	100	100	100	100	100
0.30%	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

TABLE 3

Degree of Hemolysis of the Erythrocytes of the Ox, Pig, and Sheep, Expressed in Estimated Percentages when Treated with One Drop of Guinea-Pig Serum and Varying Quantities of Inactivated Sera of Eel, Shad and Rock

Quantities of Fish Serum	Ox Number			Pig Number			Sheep Number		
	1	2	3	1	2	3	1	2	3
Eel, Anguilla chrysypa									
1 drop	100	100	100	100	100	100	50	75	75
½ drop	100	100	100	100	100	100	5	50	75
¼ drop	100	100	100	100	100	100	0	25	75
⅛ drop	75	75	100	100	100	100	0	25	10
Rock, Roccus lineatus									
1 drop	5	0	0	50	25	0	25	25	5
½ drop	5	0	0	25	10	0	5	25	5
¼ drop	5	0	0	5	0	0	5	25	5
⅛ drop	5	0	0	0	0	0	0	25	5
Shad, Alosa sapidissima									
1 drop	0	5	50	0	25	50	0	25	50
½ drop	0	5	25	0	5	50	0	25	50
¼ drop	0	5	5	0	0	50	0	25	25
⅛ drop	0	0	5	0	0	25	0	25	5

as man. The zoologically unrelated ox and pig behave similarly so far as their erythrocytes are concerned under the influence of human serum. Kolmer and Casselman's series of 10 different animals showed an entire lack of zoological association. The well-known ease with which an antisheep cell amboceptor can be prepared appears in part at least to depend on some inherent weakness of the sheep erythrocyte.

In Table 2 are shown the results of subjecting the erythrocytes of 5 different oxen, pigs and sheep to decreasing strengths of salt solution. The different animals showed considerable individual variation as to the behavior of their erythrocytes in these solutions. Thus Ox 5 and Sheep 5 had resistant corpuscles, while Pigs 2 and 3, and Sheep 1 had weak corpuscles. The average strength of solution where no hemolysis occurred with the ox corpuscles was 0.59%, with the pig corpuscles, 0.74%, and with the sheep corpuscles, 0.69%. Thus the ox cells were decidedly the most resistant, and the pig cells the least. One would expect that it might be an easy matter to prepare an anti-pig cell amboceptor. Such was not the case, though perhaps the rabbits tried were not adapted to making good amboceptor. The average point where the corpuscles of the 3 animals showed complete hemolysis in the hypotonic salt solutions are for the ox 0.39%, for the sheep 0.46%, and for the pig 0.45%, showing the ox cells to be the most resistant and the sheep and pig cells to be essentially alike. Hypotonic salt solution does not have the same effect on zoologically related animals, but the effect is not in the same direction as in the case of the action of human serum.

Table 3 shows the behavior of the erythrocytes of 3 different oxen, pigs and sheep when treated with 1 drop of guinea-pig serum and an equal and less amounts of 3 different fish sera, those of the eel, rock, and shad. The results here are quite astonishing and not at all in accordance with what one would expect from the zoological relations or from the results seen in Tables 2 and 3. The pig cells so resistant to human sera are very unstable in the presence of eel serum and the ox cells almost equally so, while sheep cells have a comparatively high degree of resistance. Rock and shad sera seem to have about the same effect on the 3 kinds of corpuscles, but rather less on the ox cells, and most on the sheep cells.

CONCLUSIONS

The ease with which the erythrocytes of the ox, pig, and sheep are broken up by the same agent bears no relation to the zoological position of the 3 animals. The erythrocytes of the ox on the whole are comparatively stable in most circumstances and appear to be well adapted as an indicator in complement fixation tests with human serum, much better than sheep corpuscles are. Sheep erythrocytes are comparatively unstable, tho relatively resistant to eel serum. Their use in complement fixation tests with human serum introduces a variable factor which is undesirable, altho it has probably little effect on the final value of the tests. Erythrocytes obtained at random from abattoir animals on the whole behave similarly from week to week, and appear to be quite as suited for hemolysis experiments, and indicators in complement fixation tests as are the corpuscles from a single animal. Ox erythrocytes seem to have better keeping qualities than the erythrocytes of the pig or sheep.

ON THE USE OF A MONILIA VACCINE IN THE TREATMENT OF SPRUE

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Sprue has been studied for years and numerous theories as to its etiology have been advanced, but without sufficient proofs to cause their acceptance by workers in tropical medicine.

Ashford has devoted much time to the study of this disease, with the result of the discovery of a monilia, which could be isolated from the tongue and feces of sprue patients and also from the tissues of persons dying of the disease. For this monilia he adopted the term Monilia psilosis. The organism and its etiologic relationship to the disease, including the production of sprue symptoms in animals, have been previously described. My studies have been serologic and I have found, using Monilia psilosis antigens, complement fixation in cases of sprue. These cases had been diagnosed and the presence of the monilia determined culturally by Ashford. During the experiments on the toxins of the Monilia psilosis evidence indicating specificity was furnished by the production of immunity in animals. This immunity was produced by the injection of killed cultures, and it was hoped from this that a vaccine might be made having a curative value.

DIAGNOSIS AND TREATMENT

In sprue the diagnosis is based on the following findings — sore tongue, excessive intestinal fermentation, light foamy diarrhea, diminution in size of liver, emaciation.

In cases in which a positive diagnosis of sprue could be made on these points, Ashford isolated Monilia psilosis from the tongue and feces of the patients, and the complement fixation test was positive in the samples of blood examined.

In sprue, numerous remedies have been tried and abandoned, but diet has remained the most reliable and successful means of treatment. Various diets are used, the effort being to secure one composed of food which would be easily assimilated and which would tend to inhibit gastro-intestinal symptoms. The milk diet, when it could be properly

carried out, gave the most favorable results, while the fruit and milk diet, the meat diet, and mixed diets have been favorably reported in some instances. Results from dietary treatment, however, are obtained only very slowly and sometimes require a period of months or even years. It has been the universal experience that it is very difficult to keep a patient on one diet for an extended period. Then, too, in certain places, particularly in tropical countries, it may be almost impossible for a patient to procure the prescribed diet.

For these reasons, constant endeavor to find a more satisfactory treatment and one requiring a shorter period of time, and especially a specific remedy, has gone on unceasingly.

Experiments demonstrated that endotoxins were probably the active toxic substances elaborated by Monilia psilosis. An emulsion of the monilia was sterilized and injected into guinea-pigs. These animals developed a relative immunity to injections of living cultures. There seemed therefore to be a possibility that a specific vaccine might be developed.

PREPARATION AND INJECTION OF THE VACCINE

Cultures of Monilia psilosis which had been isolated by Ashford from a fatal case of sprue were used. Inoculations were made on Sabouraud's medium and incubated 4 days at 37 C. An emulsion was then made of the 4 day cultures, using 1 culture in 10 c.c. of distilled water, and shaken in a machine for 2 hours, after which it was heated to 37 C. for 5 days, which allowed autolyzation to take place. After the addition of phenol (to make $\frac{1}{2}\%$ of the volume) the vaccine was sterilized at 56 C. for 1 hour. Cultures were now made to test it for sterility, and animals were injected with from 5 to 8 c.c. If the animals showed no signs of infection in 2 weeks and the cultures showed no growth, the vaccine was used. The difficulty in preparing the vaccine was due to the high thermal death point of the monilia, which is 70 C. Autolyzation was used to lower the thermal death point.

Owing to the difficulty of counting the number of monilia in the emulsion, and because of the polymorphic forms of the organism, the following method of standardization was adopted:

Ten c.c. of the emulsion was put in a Purdy graduated centrifuge tube and centrifuged at a medium speed for 5 minutes, after which a reading was taken. The amount of sediment composed of monilia should be 1% in the standard of emulsion used. The primary dose of this emulsion given was about 0.05 c.c.

There was no guide to the dosage of the vaccine thus prepared except its injection into normal persons and patients having sprue. For this purpose a primary dose of 0.05 c.c. of a 1% solution of the emulsion was used.

In the series, the dosage of the vaccine to be used was determined by its effect on normal persons and patients having sprue. Ten normal persons were injected with doses from 0.1 c.c. to 0.2 c.c. and the reactions observed; 0.2 c.c. produced a fairly severe local reaction which lasted about 4 days and subsided, without gastro-intestinal symptoms being produced. In normal persons or those suffering with other diseases doses of vaccine up to 0.6 c.c. have been administered without producing any other than a local reaction.

Five patients having sprue were injected with 0.05 c.c. to 0.1 c.c. and similarly observed. The injection of 0.05 c.c. produced a local moderate reaction and an increase of gastro-intestinal symptoms. The injection of 0.1 c.c. produced a severe reaction, the site of injection becoming deep red and inflamed for an area of 1 cm. Around this an area of erythema extended for 7.5 cm. The tongue became very sore, and the diarrhea increased with a great deal of flatulence, accompanied by a rise in temperature from 2-4 F. These symptoms were severe enough to require the patients to rest. They complained of depression and weakness.

The most marked features of the reactions noted were the increased soreness of the tongue and aggravation of gastro-intestinal symptoms.

The reaction was most severe on the 2nd day after the injection and commenced to subside about the 3rd to the 5th day. Considering the degree of local and general reactions that were produced, especially in very severe cases of sprue in which there is marked disability, it seems preferable to administer a primary dose of not more than 0.5 c.c.

The second injection was given 8 to 10 days after the first injection. The time of this administration should, it is believed, vary with the condition of the patient. The dosage given was about twice the first dose, from 0.15 c.c. to 0.2 c.c. being injected. The interval of the dosage depended on the condition of the patient, the 10 day interval being the most preferable.

In most cases the second reaction was not as severe as the first. A moderate local reaction was, however, produced which lasted 4-5 days, accompanied by an increased soreness of the tongue, slight flatulence, fever of 1-3 F. and slight diarrhea. These symptoms subsided rapidly, and after 5 days improvement was noted in the general condition of

the patients. There was a marked increased in appetite and the patients usually gained several pounds in weight. The intestinal symptoms were notably diminished.

The third injection was given 10 days after the second, from 0.5 to 0.6 c.c. being given. The reaction which followed was very mild, subsiding after about 2 days' duration. The intestinal symptoms were very slight.

The fourth injection was given about 12 days after the third and practically no reaction resulted, except a slight local induration and redness. The amount injected was 5.7 c.c. The general health of the patients by this time was greatly improved and in most cases they were gaining in weight, about 2 pounds per week. The symptoms of sprue had greatly diminished.

The fifth injection of 0.9 c.c. was given 14 days later and there was usually a slight or no gastro-intestinal reaction. The patient complained of weakness which lasted for 1 day. The diet was increased and the patients were allowed to have sugar and cereals in small amounts.

The sixth injection of 1.4 c.c. given 14 days after the 5th produced nothing but a localized reaction which lasted 2-3 days. The sprue symptoms were absent and the patient was allowed all kinds of food, including sugars and all carbohydrates. The patients were kept under observation for 2 months and no sprue symptoms noted.

HISTORY OF A TYPICAL CASE

A woman, of middle age, has had sprue for more than 2 years. Has all the typical symptoms, including severe anemia, sore tongue and intestinal disturbances. Complement fixation test for sprue, 100% positive. Monilia psilosis isolated from tongue and feces. She has been under diet treatment both in Porto Rico and the United States without material improvement.

The 1st injection of vaccine, 0.1 c.c., was followed by a very severe reaction, which kept her in bed. The local reaction following consisted in a reddened area with swelling and soreness of the arm. The constitutional symptoms were well marked and simulated an attack of acute sprue. The tongue had the increased soreness and reddening. There was an increase of flatulence and gastro-intestinal symptoms with a slight rise in temperature of 2 F. The symptoms abated after 5 days, leaving the patient with a feeling of weakness.

The 2nd injection of 0.2 c.c. was given 10 days after the 1st injection. Reaction, similar but less severe, followed, the duration being 4 days. At the termination of reaction, great improvement was noted by the patient. Gastro-intestinal and tongue symptoms were especially improved. Patient gained 9 pounds in weight in 3 weeks. The 3rd injection of 0.4 c.c. was given 18 days after the 2nd. Very slight reaction of 2 days' duration. Great improvement in general condition of patient; gained 3 pounds.

The 4th injection of 0.8 c.c. was given 10 days after the 3rd. A slight reaction followed and there was a slight flatulence which lasted 1 day. The diet of the patient was increased and there was a steady gain in weight.

The 5th injection was given 14 days later. Only a slight local reaction resulted. The patient weighs more than she has in the last 2 years and for the past 3 months since the last injection has been apparently well and has been on full diet including carbohydrates and sugars without any symptoms. Monilia psilosis cannot be found in scrapings from the tongue or feces. Complement fixation test is slightly positive.

RESULTS OF TREATMENT

Of 81 cases, 62 patients have completed the treatment. All cases were diagnosed by Ashford and Monilia psilosis was isolated from the feces. In all these cases the complement fixation test was positive. The diet was regulated so as to fit each individual case. The only medication given was to stimulate digestion. Sprue being confused with other diseases and being often complicated, a careful positive diagnosis was made in each case. Cases of sprue without complications responded most readily to the treatment. Americans responded very quickly to the treatment and required a shorter time than Porto Ricans.

Of 62 patients treated, 49 patients were discharged cured, 12 were improved, and 1 died.

Summary of Cases Treated

Cases Cured		Cases Improved		Cases Unimproved	
Cases	Number of Injections	Cases	Number of Injections	Cases	Number of Injections
10	8	1	7	1	5
11	7	2	6	Died of acute nephritis	
11	6	1	5		
10	5	7	4		
		1	3		
49 Cured	12 Improved			1 Death	

The results of the vaccine treatment are very encouraging, and all patients under treatment have shown great improvement. In noting the results of the treatment, the mental attitude of the patients has been considered to ascertain whether the injections and reactions produced a psychic improvement only. In certain cases there was a distinct abatement of sprue symptoms after the 3rd injection and instead of a diarrhea, constipation resulted. The most notable feature was the gain in weight, which was progressive from week to week. There was, in all cases, a great improvement in the general physical condition of the patient, and the disappearance of the monilia from the feces. The results of this treatment are very favorable, and it is believed that Monilia psilosis vaccine should be tried in other localities where sprue is present.

Case Histories

1.—Mrs. D., American, aged 48 years, weight 82 pounds; sick 2 years; diagnosis, sprue; all symptoms present; on a diet for one year; monilia isolated from tongue and feces; complement fixation test positive.

February 5, monilia vaccine, 0.1 c.c.; severe reaction; February 15, 0.2 c.c.; moderate reaction. March 1, 0.4 c.c.; moderate reaction; March 17, 0.6 c.c.; moderate reaction. April 3, 0.8 c.c.; no reaction; April 25, 1.2 c.c.; slight reaction; after 3rd injection patient was placed on a full diet, gained weight rapidly; all sprue symptoms had disappeared after 4th injection.

June 11, patient has no symptoms, weighs 110 pounds; monilia absent from tongue and feces.

July 1, complement fixation test negative; discharged recovered.

2.—Mrs. S., American, weighs 96 pounds; sick for nearly 1 year; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

March 23, monilia vaccine, 0.05 c.c.; moderate reaction; March 29, 0.2 c.c.; severe reaction. April 7, 0.4 c.c.; moderate reaction; April 21, 0.6 c.c.; slight reaction. May 4, 0.8 c.c.; no reaction; after 3rd injection patient was put on full diet; slight symptoms remained.

June 14, patient shows no symptoms; weighs 115½ pounds; discharged cured; feces negative for monilia.

3.—B. G., woman, Porto Rican, aged 39, weight 79 pounds; sick for over 8 months with sprue; has had previous attacks; complement fixation test positive.

March 23, monilia vaccine, 0.1 c.c.; severe reaction; March 29, 0.2 c.c.; moderate reaction. April 3, 0.4 c.c.; severe reaction. May 1, 0.8 c.c.; mild reaction; May 21, 1 c.c.; slight reaction.

June 11, all symptoms have disappeared; patient on full diet without any gastric disturbances; weight 97 pounds; vaccine was administered at too short intervals and great depression followed the reactions; discharged cured.

4.—G. A., woman, Porto Rican, weight 142 pounds; sick for over 2 years; symptoms not very severe; has not lost much weight; diagnosis, sprue; monilia isolated from tongue and feces. Complement fixation test positive.

March 27, monilia vaccine, 0.1 c.c.; moderate reaction. April 7, 0.2 c.c.; moderate reaction; April 17, 0.4 c.c.; moderate reaction; April 28, 0.6 c.c.; slight reaction. May 26, 0.8 c.c.; no reaction. June 5, 1.2 c.c.; no reaction. No sprue symptoms present; patient has been on full diet; weighs 160 pounds; discharged cured.

5.—M. P., woman, Porto Rican, aged 35, weight 78 pounds; has had sprue for several years; monilia isolated from tongue and feces; complement fixation test positive.

March 21, monilia vaccine, 0.1 c.c.; moderate reaction; March 21, 0.3 c.c.; very severe reaction. April 14, 0.6 c.c.; moderate reaction. May 1, 0.8 c.c.; slight reaction; May 21, 1 c.c.; no reaction. June 4, 1.2 c.c.; no reaction.

July 7, all sprue symptoms absent; patient on full diet without gastric disturbance; weight 96 pounds; discharged cured.

6.—Ske. E., woman, Porto Rican, aged 64, weight 67 pounds; sick for 2 years; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

April 15, monilia vaccine, 0.1 c.c.; very severe reaction. May 1, 0.2 c.c.; moderate reaction; May 15, 0.3 c.c.; moderate reaction; May 31, 0.5 c.c.; slight reaction. June 14, 0.7 c.c.; no reaction; June 29, 0.9 c.c.; no reaction.

July 14, no symptoms present; on full diet; appetite good; digestion good; weighs 79 pounds; discharged cured.

7.—A. A., man, Porto Rican, aged 19 years, weight 80 pounds; sick for 1½ years; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

May 1, monilia vaccine, 0.1 c.c.; severe reaction, fever, vomiting and diarrhea; May 11, 0.2 c.c.; moderate reaction; May 19, 0.35 c.c.; moderate reaction; May 28, 0.6 c.c.; moderate reaction. June 9, 1.2 c.c.; slight reaction; June 22, 1.4 c.c.; no reaction.

July 3, no symptoms of sprue present; on full diet; no gastric disturbance; digestion good; weighs 106 pounds; discharged recovered.

8.—V. J. A., man, Porto Rican, aged 31 years, weight 92 pounds; sick for 9 months; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

May 11, autogenous monilia vaccine, 0.05 c.c.; reaction; May 18, 0.1 c.c.; reaction; May 25, 0.2 c.c.; reaction. June 1, 0.4 c.c.; slight reaction; June 11, 0.6 c.c.; no reaction; June 25, 0.8 c.c.; no reaction; all sprue symptoms have disappeared; patient on full diet; weighs 105 pounds; discharged recovered; no gastric symptoms present.

9.—R. J., boy, Porto Rican, aged 16 years, weight 68 pounds; sick for about 3 months; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test is positive.

April 30, monilia vaccine, 0.05 c.c.; severe reaction. May 7, 0.1 c.c.; moderate reaction; May 14, 0.2 c.c.; severe reaction; May 21, 0.4 c.c.; moderate reaction. June 2, 0.6 c.c.; moderate reaction; June 13, 0.8 c.c.; no reaction.

July 18, sprue symptoms absent; no gastric symptoms; patient on full diet; weighs 79 pounds; discharged recovered.

10.—M. M., woman, Porto Rican, aged 27, weight 101 pounds; sick for 7 years with sprue symptoms; treated by a great many physicians; has been on dietetic treatment which caused a temporary improvement; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

March 21, monilia vaccine, 0.1 c.c.; reaction. April 2, 0.2 c.c.; severe reaction; April 17, 0.4 c.c.; reaction. May 1, 0.6 c.c.; slight reaction; May 15, 0.8 c.c.; no reaction; May 23, 1 c.c.; no reaction; weighs 118 pounds.

July 19, patient has no sprue symptoms; has full diet; weighs 121 pounds, which is more than she has weighed in the last 6 years; discharged recovered.

11.—P. de P., Spaniard, aged 34 years, weight 86 pounds; sick for 3 years with sprue symptoms; has been on dietary treatment which caused some improvement; monilia isolated from tongue and feces.

May 4, monilia vaccine, 0.05 c.c.; reaction; May 11, 0.1 c.c.; moderate reaction; May 18, 0.2 c.c.; moderate reaction. June 2, 0.6 c.c.; moderate reaction; June 11, 0.8 c.c.; moderate reaction. July 3, 1.1 c.c.; slight reaction; July 18, 1.3 c.c.; no reaction; patient has no sprue symptoms; has full diet; no gastro-intestinal symptoms present; weighs 94 pounds; discharged cured.

12.—A. P., Porto Rican, man, aged 55 years, weight 172 pounds; acute attacks of sprue for last 5 years; dietary treatment gave temporary relief; diagnosis, sprue; monilia isolated from tongue and feces; complement fixation test positive.

April 23, monilia vaccine, 0.1 c.c.; reaction. May 1, 0.2 c.c.; reaction; May 7, 0.3 c.c.; slight reaction; May 23, 0.6 c.c.; moderate reaction. June 5, 1.2 c.c.; moderate reaction; June 26, 1.5 c.c.; slight reaction. July 10, 1.5 c.c.; no reaction; July 26, 1.7 c.c.; no reaction; no symptoms of any kind; has very

good appetite; on full diet; weighs 184 pounds; monilia absent in feces and
scrapings of tongue; discharged recovered.

13.—A. A., woman, Porto Rican, aged 56 years, weight 130 pounds; sick
for last 3 years with acute attacks of sprue; was sent to the United States
and returned without relief; monilia isolated from feces.

May 9, monilia vaccine, 0.05 c.c.; mild reaction; May 17, 0.1 c.c.; severe
reaction; May 23, 0.2 c.c.; moderate reaction. June 1, 0.3 c.c.; moderate reac-
tion; June 12, 0.6 c.c.; moderate reaction; June 25, 0.8 c.c.; no reaction. July 9,
1 c.c.; no reaction.

July 15, no sprue symptoms present; patient weighs 137 pounds; monilia
absent from feces; discharged cured.

14.—R. M., Porto Rican, aged 48 years, weight 108 pounds; sick for 6 years
with stomach trouble; has been on dietary treatment; monilia isolated from
tongue and feces.

May 1, monilia vaccine, 0.05 c.c.; slight reaction; May 8, 0.1 c.c.; slight
reaction; May 16, 0.2 c.c.; severe reaction; May 23, 0.3 c.c.; severe reaction.
June 1, 0.4 c.c.; mild reaction; June 11, 0.6 c.c.; slight reaction; June 25,
0.8 c.c.; no reaction. July 17, 1 c.c.; no reaction; patient on full diet; no
sprue symptoms present; weighs 117 pounds; discharged recovered.

15.—V. A., man, Porto Rican, aged 45 years, weight 100 pounds; sick 1
year with typical sprue symptoms; monilia isolated from tongue and feces.

May 17, monilia vaccine, 0.05 c.c.; severe reaction; May 26, 0.15 c.c.; severe
reaction. June 4, 0.2 c.c.; mild reaction; June 13, 0.5 c.c.; moderate reaction.
July 1, 0.8 c.c.; moderate reaction; patient on full diet; no sprue symptoms;
weighs 118 pounds; discharged recovered; monilia absent from feces.

16.—*Sprue with Pellagra*. R. C., woman, Porto Rican, aged 39 years, weighs
78 pounds; sick with severe gastric disturbance, burning of the stomach, diar-
rhea; gives history of having an eruption on ears and neck; hands show evi-
dence of pellagrous eruption; could not eat meat because it would create gastric
disturbance; sprue symptoms also present; monilia isolated from tongue and
feces; complement fixation test positive; diagnosis, sprue and pellagra.

April 9, monilia vaccine, 0.05 c.c.; severe reaction; April 19, 0.1 c.c.; severe
reaction. May 1, 0.2 c.c.; severe reaction. Ulceration developed at site of
2nd injection; patient has not gained in weight, is very weak; patient is put on
a heavy protein diet and medication given to aid digestion.

May 22, monilia vaccine, 0.4 c.c.; moderate reaction; May 29, 0.6 c.c.; mode-
rate reaction. June 10, 0.8 c.c.; mild reaction. July 6, 1 c.c.; mild reaction;
patient weighs 90½ pounds; no sprue symptoms present; general condition
greatly improved; mentality improved; discharged recovered.

17.—*Sprue with Pellagra*. M. P., a woman, Porto Rican, aged 35 years, weight
78 pounds; has been sick for last 4 years; an eruption has occurred on hands
and feet every summer for last 3 years; complains of constant burning in
throat and stomach; tongue is very smooth and red; sprue symptoms also
present; monilia isolated from feces; diagnosis, sprue with pellagra; special
diet of high protein content prescribed and requested to eat beans at least
2 times a day.

April 8, monilia vaccine, 0.05 c.c.; slight reaction; April 19, 0.1 c.c.; severe
reaction. May 1, 0.2 c.c.; severe reaction; May 11, 0.4 c.c.; moderate reaction;
May 21, 0.9 c.c.; moderate reaction. June 4, 1.2 c.c.; slight reaction.

July 7, patient has slight burning in stomach; all other symptoms absent;
weighs 96 pounds; discharged recovered.

18.—*Sprue with Glycosuria.* H. M. E. B., man, American, aged 45 years; sick for past 2 years with slight attacks of sprue; complains of constant headache and dizziness; has lost very little weight; examination of urine shows 1.8% sugar present; monilia isolated from tongue and feces; complement fixation test is positive; patient was put on a diet from which carbohydrates were eliminated.

April 21, monilia vaccine, 0.05 c.c.; mild reaction; sugar in urine, 1.6%. May 9, 0.1 c.c.; severe reaction, sugar in urine, 1.8%; May 21, 0.2 c.c.; moderate reaction, sugar in urine, 1.6%; May 31, 0.6 c.c.; moderate reaction, sugar in urine, 0.936%. June 15, 1 c.c.; slight reaction, sugar in urine, 0.85%; June 28, 1.2 c.c.; very slight reaction.

July 11, patient greatly improved; no sprue symptoms present; urine is free from sugar.

July 21, improvement continues; sugar absent from urine; diet has been increased by addition of carbohydrates, including small amounts of sugar; discharged recovered.

19.—*Sprue with Tuberculosis.* O. F., man, Porto Rican, aged 21 years, weight 120 pounds; sick for over 2 years with cough and night sweats; has sprue symptoms; examination of sputum shows tubercle bacilli and feces show presence of monilia.

May 12, monilia vaccine, 0.05 c.c.; severe reaction; May 19, 0.1 c.c.; severe reaction. June 1, 0.15 c.c.; moderate reaction; June 25, 0.3 c.c.; moderate reaction. July 6, 0.4 c.c.; moderate reaction; July 17, 0.6 c.c.; slight reaction; July 28, 0.8 c.c.; no reaction.

July 30, sprue symptoms absent; has full diet; general physical condition improved; weighs 128½ pounds; tubercle bacilli still present in sputum.

20.—*Sprue with Ankylostomiasis.* J. S., man, Porto Rican, aged 31 years; weight 110 pounds; sick for 2 years with stomach trouble; during the last 3 months developed sore tongue and severe diarrhea; monilia isolated from feces; also eggs of uncinaria; all clinical symptoms of sprue present.

May 8, monilia vaccine, 0.05 c.c.; reaction; May 15, 0.1 c.c.; moderate reaction; May 22, 0.2 c.c.; moderate reaction; May 29, 0.3 c.c.; moderate reaction. June 8, 0.5 c.c.; moderate reaction; up to this time patient has not gained in weight; sprue symptoms improved; thymol given for uncinariasis.

June 20, monilia vaccine, 0.7 c.c.; slight reaction. July 3, 0.8 c.c.; no reaction; case has greatly improved; gain in weight noticeable; weighs 116 pounds; feces negative for ankylostoma; patient has full diet; no sprue symptoms present; discharged recovered.

ACTIVE IMMUNITY TO SYSTEMIC PLAGUE INFECTION

STUDIES IN PLAGUE, IV

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The problem of plague immunity has failed to lend itself to experimental methods such as have yielded successful, or at least hopeful, results in other bacterial infections.

Our knowledge concerning the mechanism of infection has been increased considerably through the recent studies of anaphylactic phenomena, particularly those dealing with the part played by certain poisons which are produced in the animal body when particular bacteria are brought into contact with substances in the serum. Friedberger's work on anaphylatoxic phenomena, as amplified by Zinsser, suggests the possibility of applying certain principles to infections which have baffled all efforts at obtaining immunity or resistance. One of the latest ideas, that of anaphylatoxins or 'proteotoxins,' broadly speaking, has thrown new light on the mechanism of bacterial infection. The whole problem of adapting these principles to specific bacterial invasions leads to questions like the following:

Does the organism, in any case, play a specific rôle in the liberation of toxic substances known as proteotoxins?

Would resistance be established for the particular organism used, if such poisons were employed to build up a proteotoxic immunity?

As to the specificity of proteotoxins, there is much to indicate that similar toxic products may be obtained by nonspecific methods, yet these results, perhaps, serve to give a broader significance to the biologic principles involved and do not detract from the importance of a mechanism which may prevail during an infection. I have stated elsewhere[1] my opinion of specificity with regard to plague. So far as the plague bacilli are concerned, I believe that they may act as a matrix for an anaphylatoxic substance and that proteotoxin obtained with B. pestis is an example of specific poison production. If this poison is specific, then resistance, when developed against it, logically ought to

[1] Jour. Infect. Dis., 1917, 20, p. 180.

be of value in warding off the particular organism which was utilized
in its manufacture. Zinsser[2] has made the first step toward demon-
strating actual resistance to bacterial proteotoxins, and it seemed to me
that no method could be better for studies in plague immunity than
one which takes into account active bodily processes approximating
the supposed mechanism of bacterial infection. This appeared to be
an ideal method of approach because the evidence indicates that the
mechanism of infectious disease is logically referable to some process
by which toxic substances are liberated in the effort of a normal body
to combat the invasive element, be it typhoid or plague bacilli. The
introduction gives rise in the body to such response that immune sub-
stances may be assumed to circulate in the blood. These substances
may or may not hold the balance of power, depending on the relation-
ship between the quantity of immune-body (sensitizer), the rate of
multiplication of bacteria, and the normal blood components. In the
method which is about to be described, we have essentially a procedure
which simulates the disease itself — the plague organisms playing an
active rôle in the production of a poison which will immunize against
the matrix. Obviously, the more toxic the poison, the better the
chances of obtaining immunity, provided the animal may be made to
survive the poison. In this manner we approximate the ideal—living
virulent bacteria as immunizing agents—which in the case of plague,
of course, is out of the question.

Whether or not the specificity of proteotoxin is a fact, if only for
the plague organism, is not within the province of this paper to discuss.
It is my contention that shock-producing substances, in all respects
confirming to what we understand as 'proteotoxins,' seem to give rise
to a definite resistance and immunity to the particular organism used
in the experiments.

The close relationship of the phenomenon of antianaphylaxis and
that of resistance to proteotoxin suggested the feasibility of applying
the facts concerning the former to a method involving apparently
similar principles. In an early work by Otto[3] on anaphylactic phe-
nomena, he showed that repeated injections of considerable amounts
of protein at short intervals resulted in a state of antianaphylaxis or
immunity to later injections. It seemed reasonable, therefore, to
assume that repeated injections of proteotoxin, if this contained speci-

[2] Jour. Exp. Med., 1914, 20, p. 387.
[3] Cited by Zinsser, Infection and Resistance, 1916, p. 362.

fic substances of the plague organism, would give rise to an immunity toward plague itself.

The results presented here are offered hesitatingly, but I feel that in spite of the limited number of experiments, the results sems to be encouraging. Until extensive data is procurable on the value of this method for man, little can be vouchsafed as to its practicability. Experiments are now being conducted on the duration of active and passive immunity; on concentration of protective substance for both active and passive treatment; on protective and curative value of serum against virulent B. pestis over varying intervals of time; and on the potency of serum when inoculated simultaneously with B. pestis; all with the hope that the final product may be utilized in those countries where plague is sufficiently prevalent to secure convincing data as to the value of this active immunity.

TECHNIC

The materials used in the production of proteotoxins were B. pestis, antipest serum from rabbits injected with plague bacteria, and normal horse serum. B. pestis was grown on plain agar slants, incubated at from 30 to 35 C. for 24 hours. The slants were of approximately uniform size, and experience with the inoculation of cultures made it possible to attain considerable accuracy for the purpose. The proportions of culture, sensitizer and normal serum were carefully worked out as discussed in earlier publications,[4] and in the following experiments the most favorable combination was utilized.

Sensitizer was obtained according to the following method: Healthy rabbits, in weight between 1200 and 1600 gm., were injected with plague bacteria emulsified in salt solution and heated for 30 minutes at 58 C. At first, intravenous injections were made, but it was found that intraperitoneal inoculation was attended with less danger of sudden death due to agglutination in the capillaries, and likewise was capable of producing a serum potent enough for the purpose. In addition, this method of injection seemed to be more easily borne by the animals. The amount of culture, given at weekly intervals, was gradually increased from 0.1 of a slant up to a whole slant, the dosage usually being 0.2, 0.4, 0.6 of a slant for the 2nd, 3rd and 4th inoculations. At times, this treatment was modified according to the reaction of the animal so that a whole slant was injected 1 week after the 3rd dose of 0.4 or 0.5 of a culture. On an average the rabbits received but 4 doses. The criterion was always serum so obtained, which was measured macroscopically for its agglutinating power. This seemed rather remarkable in its rapidity, for when the bacterial suspension received the heated sensitizer, an almost immediate flocculation ensued, and within a few minutes the bacteria formed minute clumps in a clear fluid. Within 30 minutes at body temperature, the organisms always settled to the bottom of the tube, leaving a clear fluid above.

Horse serum was obtained with sterile precautions from the jugular vein of a normal horse, collected in a tall jar and allowed to stand in a cold room for from 18 to 36 hours. The clear serum was then pipetted off and

[4] Jour. Infect. Dis., 1917, 21, p. 56.

placed in amber-colored glass-stoppered bottles. During the warmer season phenol was added as a preservative in the concentration of 0.3% but usually nothing was added to the serum which was unheated. In the preparation of proteotoxin no serum was used which exceeded 8 weeks in age.

Proteotoxin. Agar slants of B. pestis were washed off with 0.25 c.c. NaCl solution and sensitizer, heated at 56 C. for 30 minutes, was added to the emulsion in the proportion of 1.5 to 2.0 c.c. for each 1.5 slants. After 1 hour's incubation at 37 C., the material was centrifugated for 10-15 minutes, the supernatant fluid pipetted off and the bacterial sediment washed once with salt solution. Normal horse serum was then added to the sensitized complex in amounts of 10 c.c. for each 1.5 slants of B. pestis used. The final mixture was placed in large centrifuge tubes and incubated for 14 hours at 35 to 37 C. At the end of this time, the material was centrifugated for 1 hour at moderate speed. The supernatant fluid when pipetted off was clear and gave no turbidity on agitation. Tests for the presence of B. pestis were invariably negative when the centrifugation was carried out for this length of time. Prior to injection the clear fluid was heated at 56 C. for 30 minutes.

This procedure for production of toxic substance was adopted as a standard but it was impossible to adhere rigidly to the outline, particularly in regard to the temperature of incubation during the long period when normal horse serum acted on the sensitized organisms. The intensely cold weather disarranged the incubator so that at times 27 C. was the maximum temperature recorded during the entire period of incubation. Experiments which have been described elsewhere[4] show that the temperature range is quite flexible for obtaining potent proteotoxins, so that the final product is hardly affected by variations within certain limits. Deviations with respect to the sensitizer used have been previously mentioned.

Except where otherwise indicated, the intraperitoneal method of injection was used. The absorption by this route seemed to be slower and in the amount given, devoid of shock, which was usually so severe in the case of intravenously injected (marginal vein) animals that death supervened within a few minutes. Very often the rabbits thus treated died within a day or two, or at the most after a week, with severe emaciation and slow poisoning. Intraperitoneally administered, the proteotoxin caused no visible disturbance in the animal other than a slight uneasiness at first. Ten to eleven days after the last injection with proteotoxin, virulent B. pestis was administered.

The greater part of this work was carried on at the Peiyang Medical College, Tientsin, China. I wish to thank the authorities and the Director, Dr. King, for the aid which was given, and Dr. J. R. Shand, U. S. Army, for abundant serum for the experiments.

Results of Experiments

Exper. 1.—In this experiment to determine the value of repeated doses of plague proteotoxin in establishing a resistance to living virulent B. pestis intraperitoneally injected, the amount of sensitizer was 2.0 c.c. for each 3 slants of B. pestis and the normal horse serum added was 25 c.c. for the mixture. Preliminary tests showed that the proteotoxin was weak and could be tolerated in fairly large amounts when injected directly into the circulation via the marginal vein. The temperature of incubation during 1 hour of sensitization was 34 C., and that during proteotoxin formation was 30 to 34 C.

In this experiment it is interesting that 1 animal received considerably more proteotoxin than the 2nd, yet protection was just as marked in the latter.

Rabbit 2 showed a poor reaction to the 1st injection and continued loss in weight at each weekly examination made it imperative to discontinue treatment until more than 3 weeks later when the 2nd injection was given. The fact that this animal showed a striking tolerance to the inoculation with plague bacilli, suggested experiments with fewer doses of proteotoxin administered after longer intervals (see Exper. 5).

TABLE 1

RESULTS OF TESTS TO DETERMINE THE VALUE OF REPEATED DOSES OF PLAGUE PROTEOTOXIN
IN ESTABLISHING PROTECTION

Rabbit	Weight, gm.	Dose, c.c.	Route	Dose of Pest Bacilli	Result
1	1085	6.0	Intravenous	1/200 slant, 11 days after last injection of proteotoxin	Well; lived
	1052	3.0	Intraperitoneal		
	950	2.0	Intraperitoneal		
	990	3.0	Intraperitoneal		
	1025	...	Intraperitoneal		
2	1080	5.4	Intravenous	1/200 slant, 11 days after last injection of proteotoxin	Well; lived
	910	3.0	Intraperitoneal		
	870	...	Intraperitoneal		
3	1000	...	Intraperitoneal	1/200 slant	Died after 72 hours with acute septicemic plague; all organs and blood showed enormous numbers of B. pestis
4	1200	...	Intraperitoneal	1/200 slant	Died after 50 hours; postmortem as in 3

TABLE 2

RESULTS OF TESTS MADE IN STUDYING THE TOLERANCE EXHIBITED FOR LARGER DOSES OF
VIRULENT PLAGUE BACILLI

Rabbit	Weight, in gm.	Dose, in c.c.	Route	Dose of Pest Bacilli	Result
5	1090	7.2	Intravenous	1/100 slant	Well; lived
	1150	4.0	Intraperitoneal		
	1050	3.0	Intraperitoneal		
	1150	5.0	Intraperitoneal		
	1175	...	Intraperitoneal		
6	1015	2.8	Intravenous	1/100 slant	Died after 4 days and 6 hours; postmortem gave no typical signs of plague at the site of inoculation, the subcutaneous tissues or in the organs; slight congestion of the inguinal vessels was present; spleen smears showed numerous plague bacilli; blood smears were negative; peritoneum contained a large amount of exudate
	945	4.0	Intraperitoneal		
	860	3.0	Intraperitoneal		
	995	3.0	Intraperitoneal		
	1015	...	Intraperitoneal		
7	1420	...	Intraperitoneal	1/100 slant	Death after 80 hours; acute plague
8	1195	...	Intraperitoneal	1/100 slant	Death after 44 hours; typical plague infection

Rabbit 1 was killed 10 days after the test dose of plague bacilli was given, and at necropsy the liver showed a few minute fatty areas. Blood smears showed many polymorphonuclear leukocytes.

Exper. 2.—The object was to study the tolerance exhibited for considerably larger doses of virulent plague bacilli. Proteotoxin was prepared as in Exper. 1 and injections made at weekly intervals, with the dose of plague bacilli administered 11 days after the last injection.

Marked protection is evidenced here, as in Exper. 1. That the amount of material injected may have a bearing on the result is suggested, when the 2 animals are compared. Rabbit 6 lived about 1 day longer than the heaviest of the control animals, of which the weight exceeded that of the test rabbit by 400 gm. The findings after death indicated some degree of protection. Rabbit 5 was killed 9 days after it had received the plague inoculation. No lesions were demonstrable and blood smears showed enormous numbers of polymorphonuclears.

TABLE 3

Results of Tests in Studying the Tolerance Exhibited for Larger Doses of Virulent
Bacilli at a Different Incubation Temperature

Rabbit	Weight, in gm.	Dose, in c.c.	Route	Dose of Pest Bacilli	Result
9	1065	2.0	Intravenous	1/100 slant, 10 days after last injection	Well; lived
	1115	4.0	Intraperitoneal		
	1075	3.0	Intraperitoneal		
	1025	3.0	Intraperitoneal		
	1105	4.0	Intraperitoneal		
	1175	. . .	Intraperitoneal		
10	1165	4.8*	Intravenous	3/200 slant, 10 days after last injection	Death in 5 days and 9 hours; there were chronic changes in liver and spleen
	1125	3.0	Intraperitoneal		
	1010	2.0	Intraperitoneal		
	1157	4.0	Intraperitoneal		
	1175	. . .	Intraperitoneal		

* Severe shock followed the injection of proteotoxin. As controls two rabbits weighing respectively 1,200 and 1,190 gm., were given 1/100 slant intraperitoneally. These animals died within 3 days of acute septicemic plague.

Exper. 3.—The procedure was the same as in Exper. 2, with the exception that the incubation temperature during the 14-hour period ranged from 31 to 33 C. and an additional injection was made in Animal 9.

Here we see again a definite resistance on the part of treated animals. Even 3/200 of a slant, which is far in excess of a "minimal lethal" dose, failed to kill Rabbit 10 within the usual time, which for this dose would be less than 40 hours. This animal appeared perfectly healthy until the 4th day after inoculation when it became apathetic and refused food. Rabbit 9 was etherized 7 weeks after receiving the dose of B. pestis. No changes were noted in the organs. The serum was used as sensitizer in other experiments.

Exper. 4.—It seemed of interest to study the action of filtered proteotoxins in an effort to correlate shock production with the development of tolerance to living bacteria. Some writers have found that filtration of proteotoxin inhibits shock, and an experiment was made with the hope that a series of injections with filtered material would reveal an interesting relationship. The

results of Zinsser,[5] with regard to the peculiar behavior of proteotoxins were

[5] Jour. Exp. Med., 1914, 20, p. 387 and p. 582.

confirmed. A series of animals which were injected intravenously with considerable quantities of the material showed no shock, whereas controls which were treated with unfiltered material reacted severely in typical fashion. Unfortunately, it was possible to make but one injection with such filtered proteotoxin—the 1st dose—on account of an accident. The protocol serves, therefore, as an additional experiment similar to those which precede.

TABLE 4

RESULTS OF TESTS MADE TO DETERMINE THE ACTION OF FILTERED PROTEOTOXINS

Rabbit	Weight, in gm.	Dose, in c.c.	Route	Dose of Pest Bacilli	Result
13	610	2.8*	Intravenous	1/400 slant, 10 days after last injection	Well; lived
	557	3.0	Intraperitoneal		
	548	3.0	Intraperitoneal		
	620	3.8	Intraperitoneal		
	725	...	Intraperitoneal		
14	595	2.6*	Intravenous	Same as 13	Well; lived
	695	3.2	Intraperitoneal		
	695	3.0	Intraperitoneal		
	715	4.0	Intraperitoneal		
	765	...	Intraperitoneal		
15	620	3.0	Intraperitoneal	1/200 slant, 10 days after last injection	Death in 48 hours; acute plague
	580	1.8	Intraperitoneal		
	595	2.5	Intraperitoneal		
	630	3.5	Intraperitoneal		
	700	...	Intraperitoneal		
16	695	3.2	Intraperitoneal	1/100 slant, 10 days after last injection	Death, acute plague, after 44 hours
	700	3.5	Intraperitoneal		
	765	4.0	Intraperitoneal		
	740	4.0	Intraperitoneal		
	795	...	Intraperitoneal		
17	945	...	Intraperitoneal	1/200 slant	Death, acute plague, after 60 hours
18	940	...	Intraperitoneal	1/400 slant	Death, acute plague, after 80 hours

* Proteotoxin passed through Reichel filter.

Rabbits 15 and 16 became ill within 24 hours after the test dose. Necropsy revealed a severe infection. With regard to virulent B. pestis, it is impossible to draw a sharp line of demarcation between a "minimal lethal" dose and one which, if resisted, would tend to illustrate the point desired. Considerable doses were purposely given in this series of small animals in order to test the resistance toward an exaggerated infection. The animals naturally differ in their individual susceptibilities to inoculation, but it may be said that for plague, the natural mode of infection requires but a very slight amount of material which need not be introduced directly into the system as has been done in all of these experiments. Detailed studies dealing with the relation between the total amount of proteotoxin given and the resistance to varying doses of B. pestis would make this point clear.

Exper. 5.—I have mentioned that 2 injections of proteotoxin, with a considerable interval, seemed to protect a rabbit so treated. In order to study the value of such a procedure, a series of animals were given 2 injections of the material allowing 2 weeks to elapse between the doses. Proteotoxin was

prepared in the following manner: Three slants of B. pestis were washed off with 0.75 c.c. NaCl solution and 4.0 c.c. of sensitizer added. After incubating for 1 hour at 37.5 C. the mixture was centrifugated and the sediment recovered and washed once with salt solution. Twenty cubic centimeters of normal horse serum were added to the complex and the whole incubated at 37.5 C. for 14 hours. One hour's centrifugation rendered the supernatant fluid entirely clear. This was heated for 35 minutes at 56.5 C. and injected intraperitoneally.

TABLE 5

Results of Tests Made to Determine the Protective Value of Injecting the Proteotoxin at Intervals of 2 Weeks

Rabbit	Weight, in gm.	Dose, in c.c.	Dose of Pest Bacilli	Result
19	955 1012 1000	3.0 4.0 ...	1/400 slant, 10 days after last injection (intraperitoneally)	Well; lived
20	950 980 995	3.0 3.0 ...	1/400 slant, 10 days after last injection (intraperitoneally)	Well; lived
21	820 715 815	3.0 3.0 ...	1/400 slant, 10 days after last injection (intraperitoneally)	Well; lived

Controls

22	975	...	1/400 slant, intraperitoneally	Death in 5 days; acute plague
23	910	...	1/400 slant, intraperitoneally	Death in 6 days; acute plague
24	1015	...	1/400 slant, intraperitoneally	Death in 6 days; acute plague

The results indicate that a definite response is elicited in the animal body by so few as 2 injections 2 weeks apart. This interval is of distinct advantage in enabling the animal to stand up under the strain of treatment which normally occurs. One of the primary objects of this experiment was to attempt to throw some light on the question of what influence a few heated sensitized B. pestis may have in bringing about the resistance noted. A small total volume of material was given so that in the amount of proteotoxin injected the few organisms which might be present would hardly have a significant effect. In order to double-control this point, which appears to be of theoretical interest, at least, the following experiment was made.

Exper. 6.—Six slants of B. pestis were sensitized for 1 hour at 37.5 C. with 8.0 c.c. of serum, centrifugated to remove the organisms, and the sediment washed once with normal salt solution. To the organisms were then added 40 c.c. of normal salt solution and the suspension finally incubated at body temperature for 14 hours. After an hour's centrifugation, the supernatant fluid was pipetted off and heated at 56.5 C. for 30 minutes. Injections were made into the peritoneum at weekly intervals, as in the proteotoxin experiments, and living virulent plague bacilli injected via the same route 10 days after the last dose of test substance. The material which was used for this experiment appeared fairly clear after prolonged centrifugation and, in 1 instance, was distinctly turbid with B. pestis.

TABLE 6

TESTS MADE TO DETERMINE THE INFLUENCE OF HEATED SENSITIZED B. PESTIS IN BRINGING
ABOUT RESISTANCE TO INFECTION

Rabbit	Weight, in gm.	Dose, c.c.	Dose of Pest Bacilli	Result
25	1360	6.0	1/200 slant	Death, acute plague, 48 hours
	1260	4.0*		
	1190	3.4		
	1182	4.0		
	1230	...		
26	720	4.0	1/400 slant	Death, acute plague, 46 hours
	650	4.0*		
	575	3.0		
	610	6.0		
	710	...		
27	930	4.0	1/400 slant	Death, acute plague, 40 hours
	845	4.0*		
	865	5.0		
	862	5.0		
	795	...		

* Centrifuge defective; supernatant fluid heavily clouded with B. pestis.

Although few animals were used in this experiment, the results seemed to be well marked, since the amount of material injected is considerably greater than that used in any of the other experiments. In addition, we have the fact, although a mere accident, that the 2nd injection was made with material which might by itself be expected to possess some immunizing action inasmuch as it contained enormous numbers of plague bacteria which were not removable by centrifugation.

In this study no attempt has been made to determine the duration of active immunity, except in a preliminary way. A series of animals which were given several injections of the material, were to be tested with doses of living virulent plague at intervals of 1, 2, 3, 4, 5, and 6 months, respectively, after treatment. Owing to discouraging mortality among the animals, the completeness of the experiment was interfered with and it is possible to report the results for those animals only which received the test dose of plague bacteria 1 month after the last injection of proteotoxin.

Exper. 7.—The rabbits in this series received 4 intraperitoneal injections of proteotoxin obtained with B. pestis. The technic followed was the same as that in preceding experiments. The test dose of plague bacilli was administered via the peritoneum 30 days after the final injection.

The results show that rabbits treated with plague proteotoxins are definitely resistant to inoculation with B. pestis at least 1 month after treatment. Two untreated animals (31 and 32) became ill on the 2nd day following injection with B. pestis and died of typical plague in about 4 days. The 3 treated rabbits showed no illness whatever and remained alive. One of these was killed 10 days after the test dose of organisms and showed no lesions. The blood contained many polymorphonuclears.

An interesting question arises as to the mechanism of this resistance to infection with living organisms used in the preparation of the proteotoxins; also, as to the localization of this apparently specific tolerance. From the scanty material available in the course of these experi-

ments, it seems as if some support might be given to the hypothesis of a powerful leukocytic response to the substance injected. When animals were treated intraperitoneally, an injection of the test dose of plague bacteria was invariably followed by marked accumulation of polymorphonuclears in the peritoneal exudate, as well as in the blood. This was not observed in untreated rabbits. Whether or not the substance gives rise to a bacteriolytic action has not yet been determined. The question of localization of tolerance may be passed over with a few words. From the nature of plague infection it is evident that an

TABLE 7

RESULTS OF TESTS WHEN 4 INTRAPERITONEAL INJECTIONS OF PROTEOTOXIN OBTAINED WITH B. PESTIS WERE GIVEN

Rabbit	Weight, in gm.	Dose, c.c.	Dose of Pest Bacilli	Result
28	610	2.0	1/400 slant	Well; lived
	645	3.0		
	680	4.0		
	715	4.5		
	605	...		
29	650	2.0	1/400 slant	Well; lived
	715	4.0		
	725	4.5		
	765	5.0		
	740	...		
30	625	2.5	1/400 slant	Well; lived
	640	3.0		
	635	3.0		
	650	4.0		
	710	...		

Controls

31	810	...	1/400 slant	Death after 4 days; acute plague
32	790	...	1/400 slant	Death after 4½ days; acute plague

absolute immunity toward inoculation by any particular route would depend on the state of resistance developed by the treatment. Results of tests made on these points may, therefore, fail to answer the question. There is no way of controlling the efficacy of the treatment other than by the use of a very large series of animals for each type of inoculation practiced, and by further making cross injections with plague organisms, in order to determine the value of intraperitoneal injections of proteotoxin for intravenously inoculated B. pestis and that of intravenous treatment for organisms administered by a different route. The few experiments which were made on this point indi-

cate that the animal does not tolerate any considerable dose of plague when the bacteria are given intravenously after treatment with proteotoxin. Of course, since it was not possible to determine with sufficient controls whether or not the material was efficacious, it is difficult to venture an opinion.

Exper. 8.—The following table records the results of a typical experiment.

TABLE 8

RESULTS OF TESTS TO DETERMINE THE MECHANISM BY WHICH THIS RESISTANCE TO INFECTION TAKES PLACE

Rabbit	Weight, gm.	Dose, c.c.	Route	Dose of Pest Bacilli	Result
33	1115	1.0	Intravenous	1/400 slant, 11 days after last injection	Death in 5 days
	1045	3.5	Intravenous		
	980	3.0	Intravenous		
	930	2.5	Intravenous		
	965	...	Intravenous		
34	1335	3.2	Intravenous	1/400 slant, 11 days after last injection	Well; lived
	1385	4.0	Intravenous		
	1275	4.0	Intravenous		
	1444	5.0	Intravenous		
	1320	...	Intravenous		
35	1200	2.8	Intraperitoneal	1/800 slant, 10 days after last injection	Well; lived
	1197	3.8	Intraperitoneal		
	1286	4.5	Intraperitoneal		
	1026	...	Intravenous		
36	1025	3.0	Intraperitoneal	1/400 slant, 10 days after last injection	Death in 3 days; blood negative; very few organisms in spleen
	1098	3.6	Intraperitoneal		
	1172	5.0	Intraperitoneal		
	984	...	Intraperitoneal		

Controls

Rabbit	Weight, gm.	Dose, c.c.	Route	Dose of Pest Bacilli	Result
37	1408	...	Intravenous	1/800 slant	Death in 3 days; acute septicemic plague
38	1765	...	Intraperitoneal	1/400 slant	Death, acute plague
39*					

* This rabbit was treated with salt solution prepared as in experiment 6, as an added control. The animal received, in all, 16 c.c. of material in 4 intravenous injections, and the test dose of B. pestis was given 10 days after the last injection, into the ear vein. At each weekly injection the animal weighed respectively 1325, 1340, 1270, 1165 gm. and 1151 gm. at the time it received the plague inoculation. One eight-hundredth of a slant B. pestis killed the rabbit in 4 days.

SUMMARY AND CONCLUSIONS

The treatment of rabbits with proteotoxins obtained with B. pestis confers a definite resistance on the part of the animal to systemically introduced plague organisms.

There is apparent protection in at least 75% of the animals treated, taking the entire number of cases into consideration. Below a certain dose of bacteria, the protection conferred is much greater.

Combined intravenous and intraperitoneal injections of the proteo-toxin seem to be more effective than either method by itself, although intraperitoneal treatment of rabbits results in a powerful resistance to plague bacteria administered by that route.

The immunity, so far as it has been possible to determine in these experiments, is definitely marked for at least 1 month after treatment.

Injections given at intervals of 2 to 3 weeks give rise to a definite resistance to inoculation with living virulent plague bacilli.

B. pestis appears to be the matrix for a specific poisonous sub-stance of the nature of proteotoxin, and capable of inducing a specific resistance to the organism which is used in the manufacture of the toxic element.

Observations so far support the hypothesis that one of the most potent factors in the protective mechanism is that of leukocytosis.

THE STREPTOCOCCUS IN ACUTE EPIDEMIC RESPIRATORY INFECTION OF HORSES

SO-CALLED EQUINE INFLUENZA, STABLE FEVER, SHIPPING FEVER, EQUINE TYPHOID FEVER, CONTAGIOUS PLEUROPNEUMONIA, ETC.

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In the literature of equine medicine the term influenza is used in a general sense and serves to designate an acute infection, the etiology and relationship of which have not been determined. This or similar diseases known under such names as shipping fever, catarrhal fever, equine contagious pleuropneumonia, stable fever and typhoid fever, are especially frequent among horses subjected to wide variations in climatic environment, and the problems relating to their control are of great importance from the economic point of view. The clinical manifestations and pathologic changes of these infections vary greatly. In most instances, however, the symptoms are those of an acute inflammation of the upper respiratory tract, lungs, and lymph glands. The course of the infection is rapid and the outcome depends to a large degree on the extent of the process; the pulmonary type is perhaps the most fatal.

The etiology of equine influenza is still unknown. The fact that so-called influenza, strangles, pleuropneumonia, etc., are so closely associated in the epidemics that a differential diagnosis is usually impossible, has led many observers to believe that these diseases are all etiologically related. Streptococci are the only micro-organisms that have been found with any degree of regularity, but though constantly associated with these diseases, their etiologic importance remains to be satisfactorily determined. The investigations of Schütz,[1] Pfeiler,[2] Ferry,[3] and others are interesting in this regard. On the other hand, a filtrable virus has been mentioned as a causative factor in influenza and con-

[1] Arch. f. Thierheilkunde, 1888, 14, p. 172; Virchows Arch., 1887, 107, p. 356.
[2] Zeitschr. f. Infektionskr. usw. Haustiere, 1910, 8, p. 155.
[3] Vet. Jour., 1912, 19, p. 185.

tagious pleuropneumonia, and in the instance of the latter, experimental evidence of a favorable nature has been brought forward by Gaffky and Lührs.[4] So far this work has not been confirmed. Observations on the etiology and relationship of equine influenza and related epidemic infections having yielded such varying results, the possibilities for valuable research in these fields would seem promising.

During the winter of 1915-1916 a widespread epidemic of acute respiratory infections occurred among horses and mules in the United States. The epidemic began in the large concentration markets in Chicago, New York, Kansas City, and St. Louis, and rapidly spread among the shipping routes to all the smaller markets throughout the country. The manifestations of this disease were striking: the onset was sudden, usually with a chill, high fever and a mucus nasal discharge. The nasal discharge rapidly became purulent and the animal appeared profoundly exhausted. In exceptional instances gastrointestinal disturbances were noted as premonitory symptoms. As a rule the course was rapid, the attack terminating favorably unless serious complications arose. Complications, however, were frequent. Pneumonia of a lobular type, but in some instances lobar, pleuritis, empyema, abscesses, suppurative lymphadenitis, arthritis, acute nephritis, and conjunctivitis were the most common complications. It was interesting that suppurative lymphadenitis and cellulitis of the neck were more frequently observed among the younger horses suffering from the disease. When death occurred some of these more severe complications were the immediate causes.

The principal changes found after death were acute hyperemia of the mucosa of the upper respiratory tract, intestinal tract and conjunctiva; marked acute hyperplasia of the lymph-nodes throughout the whole body; acute bronchopneumonia; empyema; fibrinous pleuritis and pericarditis; moderate hyperplasia of the spleen; acute parenchymatous changes in the kidneys, liver and myocardium; the embolic suppurative nephritis and abscesses in various places. In no instance were the lungs found free from inflammatory changes which varied from a limited bronchopneumonia to involvement of entire lobes. The serous membranes were frequently involved and all types of acute arthritis were observed. Inasmuch as so little is known about the etiology of such equine epidemic diseases, a bacteriologic study was

4 Ztschr. f. Veterinäkunde, 1913, 1, Reviewed in Cornell Veterinarian, 1914, 4, p. 49.

made of this epidemic as it occurred in the Union Stock Yards in Chicago, and the results of the work form the basis of this communication. This work was furthered greatly by the interest and cooperation of Mr. A. G. Leonard, president of the Union Stock Yards and Transit Company.

TECHNIC

Cultures were made of material from the upper respiratory passages and the blood of horses at various times during the course of the infection. The material from the upper respiratory tract was collected in sterile containers and washed thoroughly through several changes of sterile normal salt solution. Stained smears were examined, and aerobic and anaerobic cultures were made on goat blood agar, ascites dextrose agar and ascites dextrose broth. All cultures were allowed to incubate 14 days before they were discarded. The bacteria obtained were identified by the usual methods. The virulence of different strains was determined and for these tests young rabbits were used.

In filtration experiments Berkefeld filters "N," and porcelain filters of the Maassen type were used. Nasal discharge or emulsion of lung tissue was macerated in a small amount of sterile salt solution before filtering. Cultures of the filtrates were made in ascites dextrose broth and ascites fluid containing a sterile piece of rabbit kidney. The ascites fluid tissue inoculations were incubated under sterile paraffin oil.

This work, which included examination of 117 horses sick or dead with the disease, yielded strikingly uniform results. In 98 horses examined before death, hemolytic streptococci were isolated from the nasal discharge in all instances. In 22 blood cultures during the course of the infection, hemolytic streptococci were found pure in 7 instances. Furthermore, these organisms were isolated from pneumonic and pleural exudates obtained during life, from the tissues about infected joints, from the urine in acute nephritis, from abscesses, iritic and conjunctival exudate, from acute nodular swellings of the extremities, and from infected lymph-nodes, in the course of the disease. Hemolytic streptococci were found regularly in infected tissues postmortem also. Other organisms such as staphylococci, green-producing streptococci, gram-negative bacilli, Bacillus subtilis and B. tetani were occasionally found in the nasal discharges. Hemolytic streptococci were the only micro-organisms found in this work with any degree of constancy. Their cultural characteristics were quite uniform. On standard blood agar plates (1 c.c. defibrinated goat blood to 9 c.c. of plain agar) the colonies were round, somewhat moist, adherent and surrounded by a clear zone of hemolysis, 2-4 mm. in diameter. In ascites dextrose broth the growth was luxuriant and the organisms settled to the bottom of the tube in the form of a white flocculent sediment. Litmus milk was acidified but not coagulated and in most instances dextrose, lactose, saccharose and salicin were fermented; many variations, however, in carbohydrate formation were observed. Anaerobic conditions were the most favorable for growth. Morphologically the organisms were variable. They were arranged in pairs, chains of pairs and single cocci, noncapsulated, and often gram-negative. The reaction to the Gram stain seemed to depend on the age of the cultures—the older cultures staining more irregularly. All the strains examined were relatively avirulent for rabbits but highly virulent for horses. Broth cultures of these organisms frequently passed through Berkefeld filters, but not porcelain filters (Maassen).

Inoculation Experiments

The results briefly described indicate that hemolytic streptococci were important elements of this epidemic disease; but other possibilities were investigated
also. Was the infectious agent present in the nasal discharge, and if so, was
it filtrable? To gain information on these points inoculation experiments were
made. It was found that a typical attack of the disease could be produced in
a normal horse by intranasal inoculation with salt solution extracts of the
fresh nasal discharge of a horse sick with the infection. In 12 instances the
nasal discharges of horses in the first stages of the infection and in 4 instances
pieces of infected lung tissue obtained from horses dead from the disease
were macerated in sterile physiologic salt solution and filtered through Berkefeld filters. The filtrates were cultured aerobically and anaerobically as well
as sprayed into the noses of normal horses. In no instance did the cultures
or inoculation experiments yield any evidence which would indicate the presence of a filtrable agent in the nasal discharge or lung tissue. It seemed logical
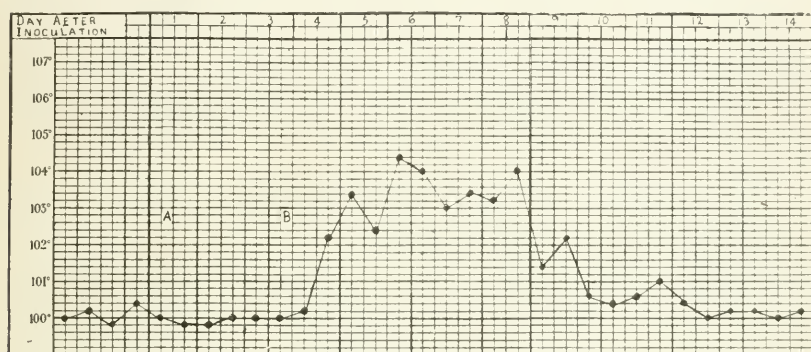
Chart 1.—The temperature curve of horse during experimental infection. *A*, horse inoculated; *B*, mucoid nasal discharge.

therefore to assume on the basis of these results that filtrable virus either was
not present in this material or it was not demonstrable by the usual methods for
the study of such viruses.

Three horses were inoculated intranasally and 1 horse intravenously with
fresh cultures of hemolytic streptococci, isolated from the nasal discharges of
horses sick with the disease. Two of the horses inoculated intranasally developed slight nasal discharges, but the general reactions following inoculation
did not justify the conclusion that they were suffering from the disease. The
3rd horse so inoculated developed a typical attack of the epidemic infection
3 days after inoculation, and its record follows:

Horse 9.—A medium sized, brown gelding, 4 years old, and in good physical
condition was inoculated intranasally, May 23, 1916, with a freshly isolated
culture of hemolytic streptococci (Strain 19X). This culture was obtained 2
days before from the nasal discharge of a horse acutely ill with the infection.
The dose used was the sediment of 180 c.c. of a 24-hour ascites-dextrose-broth
culture, which was introduced with an ordinary atomizer fitted with a long
rubber nozzle. The horse remained normal until May 26, when he suddenly
became ill, and developed a mucus nasal discharge, fever, increased pulse and
respiration. The temperature continued high for 6 days (Chart 1); on the

7th day it began to decline and gradual recovery followed and seemed complete about 18 days after the onset. There were no apparent complications other than pleuritis, the signs of which disappeared with the decline in temperature. Hemolytic streptococci were constantly present in the nasal discharge during the course of the attack, but blood cultures were sterile. The organisms isolated corresponded in every detail to the streptococci inoculated. As a control another horse was inoculated intranasally with the sterile filtrate of the nasal discharge of Horse 19X, but no signs of infections were noted as long as 1 month after the inoculation.

The horse inoculated intravenously received the bacterial sediment from 180 c.c. of 24-hour ascites-dextrose-broth culture of streptococci. Twenty-four hours later the animal developed a high temperature and all the signs and symptoms of a pleuropneumonia rapidly developed; it steadily became worse and died 8 days after the inoculation. Hemolytic streptococci were isolated from the nasal discharge, the blood, and the lung exudate obtained by puncture, before death. Postmortem examination revealed fibrinous pleuritis, acute disseminated bronchopneumonia, acute general lymphadenitis, marked acute hemorrhagic nephritis; acute parenchymatous change in the liver and myocardium. Streptococci were isolated from the lungs, kidneys, spleen, lymph-nodes and heart blood at necropsy, and found in sections of the infected tissues. A control horse was inoculated with a homologous sterile filtrate of the nasal discharge from which these streptococci were isolated, but at no time did it reveal any signs of infection.

Immunity reactions of horses, sick with this disease, with respect to streptococci have been studied in a few instances only, and the results so far are inconclusive, but this work is still in progress.

The relation of the results of this work to the clinico-anatomic characteristics of the infection is worthy of brief consideration. The acute onset, rapid course, profound exhaustion, the lobular pneumonia, the acute general hyperplasia of the lymph-nodes, and the involvement of serous membranes are all accepted characteristics of streptococcal infection. So far as this epidemic is concerned, the evidence points to hemolytic streptococci as the cause of the catarrhal inflammation of the mucous surfaces as well as of the pneumonia, and the suppurative processes. The organisms corresponded in morphology and cultural characteristics with the streptococci described by Schutz, Hel, Pfeiler and others in strangles and equine contagious pleuropneumonia, and they seemed to be highly parasitic for horses. The view that this epidemic was caused by streptococci, however, does not exclude the possibility that pneumonia and inflammations of the mucous surfaces may be caused by a variety of organisms just as in man. The streptococcus seems to bear the same pathogenic relation to the horse as to man; it is a common invader during the course of many equine infectious diseases. In the acute infections of horses in the markets this organism seems to be of primary importance, and measures for protection should be formulated accordingly. A large number of horses in transit have been inoculated with killed streptococci for purposes of protection, but the final results, which appear to be encouraging, have not yet been collected. The inoculations are being continued.

SUMMARY

During the winter of 1915-1916 an epidemic of acute infection occurred among the horses brought to the Union Stock Yards of Chicago which resembled equine influenza, or shipping fever, in its characteristics.

In a bacteriologic study of material from 117 horses sick with this disease, hemolytic streptococci were found in predominating numbers in the nasal discharges before death and infected tissues generally after death. Horses inoculated intravenously and intranasally with freshly isolated cultures of these organisms developed clinical and pathologic conditions similar to those observed in horses suffering from the epidemic disease.

The presence of pathogenic filtrable virus in material from horses sick with the infection was not demonstrated. Since hemolytic streptococci were so constantly found in predominating numbers in the nasal discharges, blood and infected tissues of horses sick with this infection, it must be concluded that they are of great importance in the etiology of the disease.

ANTIPOLLEN SERUM FOR STANDARDIZATION OF POLLEN ANTIGEN

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The work[1] on standardization of pollen extract is based either on the weight of the pollen or on a chemical analysis of the extract for its nitrogen content. These methods of standardization do not determine the antigenic strength of the extract; for, while the nitrogen content is an indicator of the amount of pollen protein in the extract, both of the methods fail to show whether the protein of the pollen is active antigenically or whether it is inert.

It seemed, therefore, that if the antigen could be standardized against antipollen serum by immunologic methods, the active antigenic power of the extract might be definitely determined. The carrying out of such tests would, of course, necessitate the employment of a potent serum. Immune rabbit serum has been prepared[2] by injecting the animals subcutaneously in the abdominal region with extracts of plant protein. Regarding this work, Goodale[3] states:

"With reference to the time required for immunization of the rabbits, no exact figures can be given. Sometimes a potent immune serum can be produced after three or four injections, but cases also occur where even after ten injections very little immunity had appeared —at times entirely failed to occur. Apparently the individuality of the animal is a factor."

Preparation of Antipollen Serum

On Jan. 7, 1915, we began the immunization of a series of rabbits, using an extract of whole pollen prepared by adding 3.64 gm. of a mixture of dried pollen consisting of equal parts by weight of the pollens of timothy, red top, June grass, orchard grass, rye, sorrel dock, daisy, maize, ragweed and goldenrod to 182 c.c. of physiologic salt solution, so that the resulting extract contained 20,000 units of pollen per cubic centimeter. The extract was preserved

[1] Noon: Lancet, 1911, p. 1572. Clowes: Proc. Soc. Exper. Biol. and Med., 1913, 10, p. 70. Koessler: Illinois Med. Jour., 1914, p. 120. Cooke: Laryngoscope, 1915, p. 108. Oppenheimer and Gottlieb: New York Med. Jour., 1915, p. 229. Cooke and Vander Veer: Jour. Immunology, 1916, p. 201.

[2] Mez and Gohlke: Cohn's Beiträge zur Biologie der Pflanzen, 1913. Physiologische-systematische Untersuchungen über die Verwandtschaften der Angio-spermen.

[3] Boston Med. and Surg. Jour., 1915, p. 201.

with 0.25% trikresol and diluted for the injections with sterile saline solution, so that the volume of each injection was 2 c.c., except the last, which was 2.5 c.c. The rabbits were injected every other day with a total of 11 injections, using the following number of units of pollen: 5, 25, 100, 500, 1000, 2000, 5000, 10,000, 20,000, 40,000, and 50,000.

Three rabbits were injected intravenously, 3 intraperitoneally, and 3 subcutaneously. Eight days after the last injection, the animals were anesthetized and bled from the carotid artery. The serum was obtained in a sterile manner, preserved with 0.25% trikresol, and stored in the ice-box at 5 C. After 6 months, the serum became anticomplementary and could no longer be used. Since then, we have preserved all antipollen serum in glycerol in a similar manner to that described in a previous paper;[4] and the serum so preserved has retained its potency and has not exhibited any anticomplementary properties.

TITRATION OF ANTIPOLLEN SERUM

In order to determine the relative potency of the different lots of serum, antibody-content titrations were made, using one-tenth the volume of the classical Wassermann reaction. The technic is indicated in Table 1, which shows the results of titrating antipollen serum from an intravenously-immunized rabbit. The serum was titrated against pollen antigen which was prepared according to the method previously described.[5] Normal rabbit serum was used for controls.

The results of the titrations showed that the serum from the intravenously-immunized and from the intraperitoneally-immunized rabbits

TABLE 1

Titration of Anti-pollen Serum, February 26, 1915

Number of Tube	Immune Serum 1:10 C.c.	Antigen		10% Complement, C.c.	0.9% Salt Sol., C.c.	Sensitized Erythrocyte Suspension, C.c.	Results
		1:25 C.c.	Gm. of Pollen				
1	0.1	0.1	0.00004	0.1	0.0	0.2	++++
2	0.09	0.1	0.00004	0.1	0.01	0.2	++++
3	0.08	0.1	0.00004	0.1	0.02	0.2	++++
4	0.07	0.1	0.00004	0.1	0.03	0.2	++++
5	0.06	0.1	0.00004	0.1	0.04	0.2	++++
6	0.05	0.1	0.00004	0.1	0.05	0.2	++++
7	0.04	0.1	0.00004	0.1	0.06	0.2	++++
8	0.03	0.1	0.00004	0.1	0.07	0.2	++++
9	0.02	0.1	0.00004	0.1	0.08	0.2	+++
10	0.01	0.1	0.00004	0.1	0.09	0.2	++
11	0.0	0.2	0.00008	0.1	0.0	0.2	—
12	0.2	0.0	0.0	0.1	0.0	0.2	—

This table shows that 0.003 c.c. was the smallest amount of the serum that gave complete fixation of complement.

Citron's standard for the strength of a reaction is used in this table; namely, complete absence of hemolysis is indicated by a 4 plus sign (++++); faint hemolysis is shown by a 3 plus sign (+++); partial hemolysis is represented by a 2 plus sign (++); while a minus sign (—) indicates complete hemolysis.

The mixtures of immune serum, antigen and complement were placed in the ice-box for 15 hours before adding the sensitized erythrocytes. The results were read after 1 hour at 37 C.

[4] Clock and Beard: Jour. Infect. Dis., 1917, 21, p. 404.

[5] Clock: Ibid., 21, p. 387.

was 10 to 15 times more potent than the serum from the subcutaneously-immunized rabbits; the smallest amounts of serum that gave complete fixation of complement being 0.003 c.c., 0.002 c.c., and 0.03 c.c., respectively. Normal rabbit serum, used as controls, failed to show any fixation of complement. In the preparation of all subsequent anti-pollen serum, the rabbits have been immunized either intravenously or intraperitoneally, and the serum has always proved to be potent.

Each lot of pollen antigen prepared has been accurately standardized against antipollen serum by the complement fixation method. The technic of titrating the antigen was shown in a previous paper.[5] This method of standardization of pollen antigen definitely determines and very accurately measures the active antigenic power of the extract against positive serum of known potency.

SUMMARY

Potent antipollen serum was prepared by immunizing rabbits intravenously or intraperitoneally with an extract of whole pollen.

Eleven injections of an increasing number of units of pollen, ranging from 5 to 50,000, produced immune serum of sufficiently high titer for use as positive serum in standardizing pollen antigen.

Antipollen serum preserved in 50% glycerol retained its potency and did not become anticomplementary after two years.

Antipollen serum, used for standardizing pollen antigen by the complement fixation method, afforded a reliable guide for determining and measuring the active antigenic power of pollen extract.

ALLERGIC PHENOMENA FOLLOWING INTRA-PORTAL INJECTIONS

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The portal blood differs from that in the general circulation in several important respects. It has been through the capillaries of the intestinal tract, and is going to the liver capillaries without aeration or contact with the tissues. During its passage through the intestinal villi it comes in contact with many cells whose function is to absorb and throw into the blood stream the various products of tryptic digestion. As I have pointed out,[1] it is probable that the proteolytic ferments are also absorbed to a certain extent and carried to the liver. It is probable, too, that the CO_2 tension in the portal blood is much greater than that in the peripheral blood because of the decreased pressure and slower circulation. This condition would tend to increase the rate of proteolysis because it has been found that under these circumstances proteolysis occurs more rapidly than when the CO_2 tension is normal.

It is not definitely known whether the protein molecules are completely broken down to the end stages of tryptic digestion before absorption or whether rather large fragments of the molecules may not be taken up as such and broken down to amino-acids by the liver before gaining entrance to the peripheral blood stream. It is well known that the liver is rich in ferments, as shown by the rapidity of autolysis postmortem under favorable circumstances. Also its position in intimate relation to the digestive tract in its embryologic development from the archenteron indicates that it is an integral part of the ferment producing mechanism of the body. With these facts in mind, it is not unreasonable to suppose that protein substances might produce atypical reactions in the allergic sense when introduced into the portal as compared with the general circulation. This would seem the more probable since heretofore it has been assumed by Abderhalden[2] and others that the liver acts as a buffer between the intestinal tract and the general circulation, and protects the latter from the entrance of foreign protein which, when split, would be a potential source of protein poison.

[1] Jour. Am. Med. Assn., 1915, 65, 524.
[2] Beitr. z. klin. Infektionskr. u. Immunitätsforschung, 19.

Manwaring,[3] in an attempt to locate the seat of the reaction in anaphylactic shock, came to the following conclusion:

The acute anaphylactic shock in dogs is an explosion like autointoxication originating in the liver and digestive tract. This autointoxication is modified, restricted, and overcome by a more or less powerful antianaphylactic mechanism which has its origin in part at least in other organs. Further, the present state of our knowledge permits us to put forward working hypotheses regarding the chemical nature of the autointoxication.

Some evidence has been brought forth to show the possibility of sensitization and of producing shock phenomena through the portal system by feeding certain proteins.

Thus, La Roche, Richet and St. Girons[4] sensitized guinea-pigs by feeding cow's milk and then shocked them by intradural injections. It took 12 days for the anaphylactic state to develop; none of the pigs died.

Richet,[5] by feeding krepetin to dogs sensitized them so that death resulted with shock when intravenous injections were made that ordinarily were harmless. Also, after injecting a dose of sufficient size to be on the borderline of toxicity, toxic symptoms resulted on feeding krepetin, but in no case did death result. Vaughan[6] has shown that rabbits may be sensitized by injecting the blood of rabbits which have previously had egg-white injected into the stomach or rectum. This, he thinks, shows that the egg-albumin may be absorbed in part at least unchanged. However, this conclusion hardly follows from the experiments detailed, because the protein molecule may be split to a considerable extent (i. e. till it no longer gives a biuret reaction) and still retain its power to sensitize. Chiray[7] has studied the effects of the administration of heterologous proteins. The intravenous injection of a small amount of egg-white in rabbits causes a transitory albuminuria in 30 minutes. The albuminuria appears much later when the injection is made into the portal vein. Subcutaneous injections cause an albuminuria which appears later, is less marked, and persists longer than when intravenous injections are made. He came to the conclusion also that alimentary albuminuria in rabbits following rectal injections of egg-albumin is not due to poisons resulting from the splitting of the protein molecules, but to the absorption and elimination of the unbroken protein molecule, which is a foreign and poisonous body. He also found that casein may in some instances be found in the urine when a milk diet is given. Wells[8] found that egg-white subjected to tryptic digestion lost its sensitizing properties as the stage of digestion progressed.

It seemed of interest to study the reaction after intraportal protein injections to determine if possible whether or not animals could be sensitized by portal injections of foreign proteins, and, if sensitized by this type of injection, whether or not anaphylactic shock could be

[3] Ztschr. f. Immunitälsf. u. Exper. Therap., 1910, 8, p. 1.
[4] Compt. Rend. Soc. Biol., 1911, 70, p. 169.
[5] Ann. de l'Inst. Pasteur, Par., 1911, 15, 580.
[6] Protein Split Products, 355.
[7] Thèse de Paris, 1906, Jahresbericht d. Tierchemie, 1907, 36, p. 805.
[8] Jour. Infect. Dis., 1908, 5, p. 449.

produced by the same route; further, whether an animal could be sensitized by portal injection and shocked by peripheral intravenous injection, and sensitized by peripheral and shocked by intraportal injection; also, if differences in the sensitizing and shock doses necessary to produce the reaction could be detected, when using the portal or peripheral circulation as the place of injection.

Young guinea-pigs as nearly as possible of uniform size were selected for the reason that these animals are responsive to protein injections both as to sensitization and as to characteristic shock phenomena.

Human blood serum was used as the antigen for 2 reasons. First, because it could be easily obtained fresh; second, in order to learn more about its action as an antigen concerning which but little is found in the literature. The sensitizing dose was uniformly 0.002 c.c. of a fresh 1:100 dilution of serum in physiologic salt solution, in the cases in which the injections were made intravenously. When the sensitizing dose was given intraperitoneally this same amount was given in some cases, in others 0.005 c.c. was used. The shock doses were given intravenously in all, and were usually composed of the whole serum. They varied in size in order that the effects of varied amounts might be studied.

Injections were made with a fine needle attached by a short rubber coupling to a pipet, graduated to one-tenth c.c.; rubber tubing on the other end of the pipet allowed blowing after the needle was placed in the vein. This was an advantage since it reduced manipulation to the minimum, which is very desirable when working with such fine structures as the mesenteric veins of guinea-pigs under light ether anesthesia. A large vein was selected and a ligature of cotton thread placed around it, but not tied. The vein was punctured just below the ligature and the injection made, the amount being read off on the graduated pipet. The needle was removed and the ligature tied. Peritonitis did not develop. Peripheral intravenous injections were made in the usual way into the jugular vein.

I have grouped the animals used in these experiments into 6 groups. As a preliminary experiment 0.002 c.c. was injected intraportally into a guinea-pig. The animal died suddenly with all the appearances and findings of anaphylactic shock. This experiment was suggestive; nothing similar occurred in subsequent injections, the nearest approach to anaphylactic phenomena being a roughening of the coat and emptying of bladder and rectum when a dose of this size was given intraportally; recovery followed in all cases. With 0.005 c.c. instead of 0.002 c.c. only slight shock symptoms resulted.

Group 1.—Six pigs (3-8) received 0.002 c.c. of human serum intraperitoneally and 17 days later 0.2 c.c. of the same serum by intraportal injection. Pig 3 showed slight shock symptoms but 4 and 5 showed no effects of the injection. The other 3 pigs of this group were injected intravenously into the jugular vein with the same dose, and all showed slight shock symptoms.

Group 2.—Five pigs (9-13) sensitized intraportally with 0.002 c.c. and shocked with various doses intrajugularly with the exception of Pig 13, which received both the doses intraportally. Pigs 9, 10 and 11 received 0.5 c.c., 0.4 c.c., and 0.3 c.c. respectively; 9 and 10 died in 4 minutes, 11 died in about 5 minutes. Pig 12 received 2 c.c. peripherally and gave only slight symptoms. Pig 13, which received the shock dose as well as the sensitizing dose intraportally, showed no effect even though the shock dose of 0.5 c.c. was equal to nearly

twice the minimum fatal dose necessary to kill with injection into the peripheral circulation.

Group 3.—Five pigs (14-18) were sensitized intraperitoneally with 0.005 c.c. After 17 days Pig 14 received 0.4 c.c. intrajugularly and died in 2 minutes. Pig 15 received the same dose intraportally and showed no effect. Pig 16 received 0.6 c.c. intraportally with negative result. Pig 17 received 1 c.c. intraportally and showed marked signs of anaphylaxis but recovered. Pig 18 received 1.5 c.c. intraportally and died in 20 minutes.

Group 4.—In order to test the effect of this type of sensitization and shock injection on passive anaphylaxis, 4 pigs were injected with the blood serum of Pig 19, Group 3, which had been sensitized by the intraperitoneal injection of 0.005 c.c. of human serum 16 days previously; 0.2 c.c. was the dose for passive sensitization, and Pigs 20 and 21 were sensitized passively intraperitoneally, while 22 and 23 were sensitized passively intraportally. The shock dose, administered one hour later, was uniformly 1 c.c. of whole human serum. Pig 20, sensitized passively intraperitoneally and shocked by intrajugular injection, showed marked dyspnea and itching, but recovered completely. Pig 21, sensitized passively intraperitoneally and shocked intraportally

TABLE 1

Summary of Experiments

Group	Guinea-Pig	Sensitiz-ing Dose in C.c.	Injection	Shock Dose in C.c.	Injection	Result
I	1	0.002	Intraportal	...	....	Died 3 min.
	2	0.005	Intraportal	...	....	Slight shock symptoms
	3	0.002	Intraperitoneal	0.2	Intraportal	Slight shock
	4	0.002	Intraperitoneal	0.2	Intraportal	No shock
	5	0.002	Intraperitoneal	0.2	Intraportal	No shock
	6	0.002	Intraperitoneal	0.2	Intrajugular	Slight shock
	7	0.002	Intraperitoneal	0.2	Intrajugular	Slight shock
	8	0.002	Intraperitoneal	0.2	Intrajugular	Slight shock
II	9	0.002	Intraportal	0.5	Intrajugular	Death 4 min.
	10	0.002	Intraportal	0.4	Intrajugular	Death 4 min.
	11	0.002	Intraportal	0.3	Intrajugular	Death 5 min.
	12	0.002	Intraportal	0.2	Intrajugular	Slight shock
	13	0.002	Intraportal	0.5	Intraportal	No effect
III	14	0.005	Intraperitoneal	0.4	Intrajugular	Death 2 min.
	15	0.005	Intraperitoneal	0.4	Intraportal	No effect
	16	0.005	Intraperitoneal	0.6	Intraportal	No effect
	17	0.005	Intraperitoneal	1.0	Intraportal	Marked reaction, recovery
	18	0.005	Intraperitoneal	1.5	Intraportal	Death 20 min.
IV	20	0.002	Intraperitoneal	1.0	Intrajugular	Marked shock, recovery
	21	0.002	Intraperitoneal	1.0	....	Slight shock
	22	0.002	Intraportal	1.0	Intrajugular	Marked shock, recovery
	23	0.002	Intraportal	1.0	....	Slight shock
V	24	0.002	Intraportal	0.4	Intrajugular	Marked reaction, recovery
	25	0.002	Intraportal	0.5	Intraportal	No effect
	26	0.002	Intraportal	1.0	Intraportal	Slight reaction
	27	0.002	Intraportal	1.5	Intraportal	Marked reaction
	28	0.005	Intraperitoneal	0.4	Intrajugular	Death 5 min.
	29	0.005	Intraperitoneal	0.5	Intraportal	Slight irritation
	30	0.005	Intraperitoneal	0.8	Intraportal	Death 20 min.
	31	0.005	Intraperitoneal	1.0	Intraportal	Death 30 min.
Controls	32	1.5	Intraportal	...	....	Appeared toxic, recovered
	33	1.5	Intraperitoneal	...	....	Death 4 min.
	34	1.15 Salt Sol.	Intraportal	...	....	No effect

with the same dose as used in Pig 20, showed only slight irritation. Pig 22, sensitized intraportally passively and shocked intrajugularly, showed marked dyspnea and itching, while Pig 23, which was sensitized passively intraportally and shocked intraportally, showed only slight itching.

Group 5.—A repetition of the experiments in Groups 1 and 2, the results being the same. Pig 24, corresponding to Pig 10, differed in that it recovered after a severe reaction while Pig 10 died. Pig 25 duplicates Pig 13, showing no reaction. Pigs 26 and 27 correspond to 17 and 18 except that the pigs were sensitized by intraportal injection and the reaction was less violent. Possibly this difference is due to a difference in the human serum used in the experiments. Pigs 28 to 31 are duplicates of 14 to 17 of Group 3. Pig 28 was killed in 5 minutes with a shock dose of 0.4 c.c. intrajugularly. A slightly larger dose injected intraportally in Pig 29 caused slight reaction. However, double the dose intraportally in Pigs 30 and 31 caused death in 20 and 30 minutes, respectively.

As control 1.5 c.c. of human serum was injected intraportally and intrajugularly into nonsensitized pigs. Pig 32, receiving the portal injection, appeared toxic but recovered. Pig 33, receiving the intrajugular injection, died in 4 minutes. Pig 34 received 1.5 c.c. of normal salt solution intraportally with no effect.

Necropsy was made of every pig that died, and in all cases the typical picture of acute anaphylactic shock was found—the lungs were distended, and there were subserous hemorrhages and congestion of the kidneys.

The results show that guinea-pigs may be sensitized and shocked by injections into the portal circulation. Larger doses, however, are necessary to produce the typical anaphylactic reaction than when the injections are made into the peripheral circulation. Apparently there is a protective action against foreign protein somewhere in the portal system, whether it be in the portal blood itself or whether it is a function of the liver cells. Since the shock dose must be larger to produce the same results several possibilities present themselves. First, the ferment content of the portal blood may be such that protein is rapidly split beyond the toxic stage, and hence toxic substances are not present in sufficient amount at any one time to produce the characteristic reaction. Secondly, the products of the protein molecule may unite with other products of tryptic digestion, thus masking their toxic action. Thirdly, the liver cells may unite with and detoxicate the products, thus protecting the body from their deleterious effects. Fourthly, the sluggishness of the portal circulation with normally active proteolytic digestion of the protein introduced may result in the protein toxic radicals being split beyond the toxic stage before they come in contact with the general circulation, the response to which is the characteristic phenomena of anaphylactic shock. Finally, a certain amount of the injected protein may be absorbed or bound by the liver cells before

splitting occurs, leaving thus only a portion available for digestion and formation of anaphylatoxin.

The possible influence of an increased CO_2 tension causing an increased proteolysis due to stimulation of the catalytic action of the ferments by increasing the H-ion content of the blood is suggested by the interesting work of Bradley on intracellular ferment activity.

<div align="center">CONCLUSIONS</div>

Guinea-pigs may be sensitized by intraportal injections of human serum.

Anaphylactic shock may be evoked after the usual latent period by subsequent intraportal injection.

Guinea-pigs may be sensitized by intraportal injections and shocked by peripheral intravenous injections of human serum.

Conversely, guinea-pigs may be sensitized by intraperitoneal injections and shocked by intraportal injections.

Larger doses are necessary to produce anaphylactic shock in guinea-pigs when the shock dose is administered intraportally, irrespective of the method of administration of the sensitizing dose.

Passive anaphylaxis may be transmitted to guinea-pigs by way of the portal system and shock may be produced by portal or peripheral injections. Here again, however, larger doses seem necessary to produce shock when administered by the portal system.

Human serum sensitizes and produces anaphylactic shock in the guinea-pig. It is, however, not without toxic effect on nonsensitized guinea-pigs as shown by the fact that 1.5 c.c. intrajugularly caused death in 4 minutes with symptoms of anaphylactic shock, the same amount introduced intraportally producing toxic symptoms but not death.

MICROSCOPIC DEMONSTRATION OF COCCI IN THE CENTRAL NERVOUS SYSTEM IN EPIDEMIC POLIOMYELITIS

Ludvig Hektoen, George Mathers, and Leila Jackson

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After reviewing recent observations on a coccus found in the central nervous system in epidemic poliomyelitis,[1] one of us wrote as follows[2] :

"To conclude—the exact significance of this coccus in epidemic poliomyelitis cannot be determined now. The number of cases studied for its presence is too small to permit the conclusion that it occurs constantly in the disease or any form of the disease; in the few instances in which injections of culture have resulted in a condition indistinguishable from what is accepted as poliomyelitis in the monkey, the possibility that another and more important microbe may have been present cannot be excluded; the true poliomyelitic nature of the very interesting lesion caused by the coccus in rabbits has not been confirmed by proper tests on monkeys; and we lack also the results of extended immunization experiments. In any event a most interesting coccus has been found that merits study for its own sake as well as on account of the close relation its brief history bears to poliomyelitis."

In order to determine so far as possible whether coccal forms are demonstrable microscopically in the central nervous system in poliomyelitis as it occurs in different places, we obtained from various sources fixed poliomyelitic material, mostly pieces of spinal cord, which we have studied, and we now wish to make a brief report of the results.

On account of the variation in the time after death when the tissues had been fixed as well as on account of the different fixatives employed, uniform results have not been obtained with all the methods of staining that were used. For demonstration of cocci the gram stain was the most satisfactory, but methylene blue and

[1] Mathers: Jour. Am. Med. Assn., 1916, 67, p. 1019; Jour. Infec. Dis., 1917, 20, p. 113. Rosenow, Towne and Wheeler: Jour. Am. Med. Assn., 1916, 67, p. 1202. Nuzum and Herzog: Ibid., p. 1205; Nuzum, ibid., p. 1437.

[2] Hektoen: Recent Investigations on the Bacteriology of Acute Poliomyeltis, Boston Med. and Surg. Jour., 1917, 176, p. 687.

TABLE 1

Results of the Study of Material from 57 Cases of Epidemic Poliomyelitis

Number	Age	Duration of Illness	Paralysis	Autopsy; Time of after Death	Changes in Cord and Brain	Bacteria in Sections	Source of Material
1	17 mo.	Several days, exact time unknown	No characteristic symptoms	17 hours	Marked ganglion cell destruction, round cell infiltration, hyperemia in cord	Medium sized and minute diplococci. Fig. 1	J. H. Wright, Massachusetts General Hospital, Boston
2	5 wk.	Few hours	No characteristic symptoms	19 hours	Extreme changes; marked neurophagocytosis; round cell infiltration and destructive changes throughout gray substance of cord	Medium sized cocci and diplococci	J. H. Wright, Massachusetts General Hospital, Boston
3	18 yr.	3 days	Paralysis	28 hours	Marked round cell infiltration, ganglion cell degeneration, hyperemia in cord and stem. round cell perivascular infiltration	Small and medium sized cocci in gray substance of cord	H. E. Robertson, Univ. of Minnesota, Minneapolis
4	35 yr.	6 days	Paralysis	10 hours	Subacute changes, neurophagocytosis and perivascular round cell infiltration being the most striking changes	Very few single cocci and diplococci. Fig. 2	H. E. Robertson, Univ. of Minnesota, Minneapolis
5	11 yr.	4 days	Paralysis	4½ hours	Extreme subacute poliomyelitic changes in cord	Cocci in pairs and short chains; very small and variable in size. Fig. 3	H. E. Robertson, Univ. of Minnesota, Minneapolis
6	3 yr.	11 days	Paralysis	13 hours	Moderate subacute poliomyelitic changes in cord	Cocci in pairs, some large, some small	H. E. Robertson, Univ. of Minnesota, Minneapolis
7	18 yr.	10 days	Paralysis	13 hours	Marked changes in the gray substance of the spinal cord, typical of poliomyelitis	Cocci, single and in pairs, of varying size	H. E. Robertson, Univ. of Minnesota, Minneapolis
8	16 mo.	33 days	Paralysis	About 12 hours	Moderate, subacute changes; small capillary hemorrhages especially numerous, diffuse round cell infiltration	A few cocci in pairs, variable in size	D. R. Gurley, Department of Health, New York City
9	2½ yr.	6 days	Paralysis	24 hours	Marked changes, poliomyelitic in nature, especially in upper end of cord	Small cocci in pairs and singly. Fig. 4	D. R. Gurley, Department of Health, New York City
10	7 mo.	7 days	Paralysis	Same day as death	Characteristic changes of poliomyelitis in dorsal cord	Cocci in pairs and singly; not numerous	D. R. Gurley, Department of Health, New York City
11	19 mo.	36 days	Paralysis	Same day as death	Marked poliomyelitic changes in the gray substance of cord	Small cocci in pairs and singly. Fig. 5	D. R. Gurley, Department of Health, New York City
12	32 yr.	5 days	Paralysis	Same day as death	Extreme poliomyelitic changes in the gray substance of cord	Cocci, quite numerous	D. R. Gurley, Department of Health, New York City
13	12 mo.	7 days	Paralysis	Day following death	Infiltration about blood vessels is only noteworthy change	Cocci, variable in size, in pairs and singly	D. R. Gurley, Department of Health, New York City
14	9 yr.	?	Paralysis	2½ hours	No poliomyelitic change	No bacteria demonstrable	F. B. Mallory, Boston City Hospital, Boston
15	1¼ yr	?	Paralysis	2 hours	No changes characteristic of poliomyelitis	No bacteria observed	F. B. Mallory, Boston City Hospital, Boston
16	Infant	?	Paralysis	9½ hours	Characteristic changes of poliomyelitis, acute in type; no neurophagocytosis	Cocci in pairs and groups. Fig. 6	F. B. Mallory, Boston City Hospital, Boston
17	10 mo.	?	Paralysis	12 hours	Acute poliomyelitic changes	Cocci in pairs and singly, large and small	F. B. Mallory, Boston City Hospital, Boston

No.	Age		Symptom	Time after death	Pathological changes	Bacteria	Source
18	2 yr.	?	Paralysis	4 hours	Marked acute changes characteristic of poliomyelitis	Cocci in pairs and short chains. Fig. 7	F. B. Mallory, Boston City Hospital, Boston
19	15 yr.	?	Paralysis	12 hours	Perivascular and diffuse infiltration, especially in the anterior horns of cord	Cocci in pairs and singly	F. B. Mallory, Boston City Hospital, Boston
20	1½ yr	?	Paralysis	17¾ hours	Sections do not stain well. Diffuse infiltration of spinal cord and marked perivascular infiltration especially of vessels of anterior fissure	Cocci in pairs and singly. Fig. 8	F. B. Mallory, Boston City Hospital, Boston
21	1½ yr.	?	Paralysis	3 hours	Hemorrhages and cell infiltration	Cocci in pairs and singly	F. B. Mallory, Boston City Hospital, Boston
22	1½ yr.	?	Paralysis	8 hours	Marked perivascular changes, and small areas of diffuse infiltration in gray substance of cord; many hemorrhages; neurophagocytosis	Cocci in gray substance of cord. Fig. 9	F. B. Mallory, Boston City Hospital, Boston
23	9 yr.	?	Paralysis	10 hours	Hemorrhages and infiltration	Cocci, singly and in pairs	F. B. Mallory, Boston City Hospital, Boston
24	9 yr.	?	Paralysis	50 minutes	Marked diffuse and perivascular infiltration and hemorrhages in gray substance of cord	Cocci, in pairs and singly	F. B. Mallory, Boston City Hospital, Boston
25	? (81 cm.)	?	Paralysis	3½ hours	Marked meningeal changes and extreme infiltration of gray substance of cord	Cocci, small and large	F. B. Mallory, Boston City Hospital, Boston
26	3½ yr.	?	Paralysis	45 minutes	Changes pronounced, especially infiltration	Cocci, small and large	F. B. Mallory, Boston City Hospital, Boston
27	9 mo.	?	Paralysis	5½ hours	Marked changes, with pial exudate	Cocci, in pairs and singly. Fig. 10	F. B. Mallory, Boston City Hospital, Boston
28	3½ yr.	?	Paralysis	55 minutes	Changes in the gray substance of a mild type	Small cocci	F. B. Mallory, Boston City Hospital, Boston
29	25 yr.	?	Paralysis	2½ hours	Marked poliomyelitic changes in cord; pial exudate containing large numbers of leukocytes	Small and large coccus forms	F. B. Mallory, Boston City Hospital, Boston
30	20 mo.	5 days	Paralysis	Soon after death	Marked infiltration and numerous hemorrhages in gray substance of cord	Small and large forms of cocci. Fig. 11	J. A. Kolmer, Univ. of Pennsylvania, Philadelphia
31	4 yr.	2 days	Paralysis	Soon after death	Moderate infiltration and a few hemorrhages in gray substance of cord	Small and large cocci	J. A. Kolmer, Univ. of Pennsylvania, Philadelphia
32	8 yr.	4 days	Paralysis	Soon after death	Patchy infiltration of gray substance as well as perivascular changes	Large and small cocci	J. A. Kolmer, Univ. of Pennsylvania, Philadelphia
33	3½ yr.	9 days	Paralysis	Soon after death	Marked diffuse infiltration of gray substance throughout which are scattered denser collections of cells	Large and small cocci	J. A. Kolmer, Univ. of Pennsylvania, Philadelphia
34	2 yr.	7 days	Paralysis	4 hours	Extensive changes in the cord and meninges	Large and small cocci. Fig. 12	G. W. McCoy, U. S. Public Health, Washington, D. C.
35	40 da.	4 days	Paralysis	5 hours	Perivascular and diffuse infiltration especially of anterior horn with minute abscesses; satellitosis	Medium sized cocci forms	G. W. McCoy, U. S. Public Health, Washington, D. C.
36	2 yr. 11 mo.	3 days	Paralysis	11 hours	Marked infiltration of gray substance of cord	Large and small cocci. Fig. 13	G. W. McCoy, U. S. Public Health, Washington, D. C.
37	2½ yr.	13 days	Paralysis	3 hours	Mild diffuse and perivascular infiltration of gray substance of cord	Diplococci	McKenzie, Presbyterian Hospital, New York City
38	5½ yr.	?	Paralysis right leg and both arms	7 hours	No characteristic changes in spinal cord	No bacteria	McKenzie, Presbyterian Hospital, New York City

TABLE 1—*Continued*

RESULTS OF THE STUDY OF MATERIAL FROM 57 CASES OF EPIDEMIC POLIOMYELITIS

Number	Age	Duration of Illness	Paralysis	Autopsy; Time of after Death	Changes in Cord and Brain	Bacteria in Sections	Source of Material
39	5 yr.	4 days	Paralysis	78 hours	Moderate acute changes; neurophagocytosis	Cocci, small and large	McKenzie, Presbyterian Hospital, New York City
40	2½ yr.	?	Paralysis	30 hours	Hemorrhages and early infiltration	Cocci, large and small	McKenzie, Presbyterian Hospital, New York City
41	17 mo.	?	Paralysis neck and arms	13 hours	Marked changes; hemorrhages, infiltration and neurophagocytosis in brain and cord	Cocci in cord	McKenzie, Presbyterian Hospital, New York City
42	14 mo.	?	Facial paralysis	14 hours	Moderate inflammatory changes in gray substance of cord	Cocci, large and small	McKenzie, Presbyterian Hospital, New York City
43	3 yr.	?	Paralysis 9th, 10th and 12th nerves	12 hours	Very slight changes	Cocci in gray substance of cord	McKenzie, Presbyterian Hospital, New York City
44	3 yr.	3 days	Paralysis cerebral type	6 hours	Marked perivascular changes in brain and cord; hemorrhages also	Cocci in brain and cord	McKenzie, Presbyterian Hospital, New York City
45	9 mo.	?	Paralysis	20 hours	No changes	No bacteria demonstrable	McKenzie, Presbyterian Hospital, New York City
46	7 yr.	2 days	Paralysis	18 hours	Hemorrhagic changes predominate	Cocci in brain and cord	McKenzie, Presbyterian Hospital, New York City
47	5½ yr.	?	Cerebral type	15 hours	Marked infiltrative changes in brain and cord	Cocci in cord	McKenzie, Presbyterian Hospital, New York City
48	6 yr.	5 days	Respiratory paralysis	4 hours	Marked changes	Cocci in brain and cord	Mathers, Memorial Institute of Infectious Diseases
49	6 yr.	3 days	Respiratory paralysis	12 hours	Marked infiltrative changes in gray substance of cord	Cocci in cord	Mathers, Memorial Institute of Infectious Diseases
50	26 yr.	7 days	General paralysis	1 hour	Changes typical of poliomyelitis	Cocci in cord and brain	Mathers, Memorial Institute of Infectious Diseases
51	24 yr.	5 days	Respiratory paralysis	1 hour	Marked poliomyelitic changes	Cocci in brain and cord. Fig. 14	Mathers, Memorial Institute of Infectious Diseases
52	2 yr.	9 days	Extremities paralyzed	2 hours	Extreme infiltrative changes, especially in gray substance of cord	Cocci in brain and cord	Mathers, Memorial Institute of Infectious Diseases
53	6 mo.	9 days	Respiratory paralysis	8 hours	Moderate infiltration and hemorrhages in spinal cord	Cocci in brain and cord	Mathers, Memorial Institute of Infectious Diseases
54	6 yr.	6 days	Cerebral type	2 hours	Marked infiltrative changes, some satellitosis	Cocci, large and small in cord	Mathers, Memorial Institute of Infectious Diseases
55	14 yr.	5 days	Respiratory paralysis	3 hours	Moderate infiltration, diffuse and perivascular; hemorrhages in gray substance of brain and cord	Cocci, large and small in cord	Mathers, Memorial Institute of Infectious Diseases
56	2 yr.	4 days	Extremities involved	1 hour	Marked infiltration and hemorrhagic changes in cord	Cocci, large and small in cord. Fig. 15	Mathers, Memorial Institute of Infectious Diseases
57	Material from epidemic of 1906 in Norway				Extensive poliomyelitic changes in gray substance of cord	Cocci, large and small	F. Harbitz, Christiania, Norway

eosin and polychrome methylene blue and eosin also proved useful. The pieces were embedded in paraffin; the sections were from 5-10 microns thick.

In all, material from 57 cases has been studied (Table 1). Except in a few instances changes characteristic of epidemic poliomyelitis were present, most markedly in the gray matter, particularly the anterior horns, and in some cases, but to a much less extent, also in the membranes. The changes were hemorrhages, edema, and cellular infiltration, especially about the blood vessels; in most cases the perivascular infiltration was marked, and often associated with more diffuse infiltrations in the gray matter as well as dense focal accumulations of cells. Typical neurophagocytosis and extensive destruction of ganglion cells were present, but not in all the specimens; in a few cases the ganglion cells appeared to have been singled out for attack.

Definite coccal forms were found rather easily in sections showing typical poliomyelitic changes (Table 1 and Plates 1-3). They were single, oftener in pairs, occasionally in small clumps, usually outside but also within cells. They were located in the gray matter, in the walls of the blood vessels, in perivascular and other infiltrates, and in hemorrhagic areas; they were found also in meningeal infiltrations. These cocci correspond in general, so far as shape and size and staining by Gram's method are concerned, with the cocci recently isolated in cultures of the central nervous system in epidemic poliomyelitis. This statement is particularly applicable to Cases 48 to 56, Table 1, in which pure cultures of the coccus in mind were obtained by Mathers[1] from the brain and cord. The cocci in the sections may vary in size, and larger and smaller forms may occur together. We did not find any other microbic forms than those described, and we found no such forms at all in tissues which did not show any changes.

SUMMARY

The cord and other parts of the central nervous system of about 50 instances of epidemic poliomyelitis, occurring in different parts of the country, have been found to contain in stained sections, cocci which look quite like the cocci that may be grown in cultures from the brain and cord in poliomyelitis. This result indicates that such cocci occur constantly in the central nervous system in epidemic poliomyelitis, and that their presence here is not explainable as due to accident or contamination.

PLATES 1, 2, 3

Magnification 1000 ×. For details of the cases see Table 1.

Fig. 1.—Case 1.   Fig. 9.—Case 22.

Fig. 2.—Case 4.   Fig. 10.—Case 27.

Fig. 3.—Case 5.   Fig. 11.—Case 30.

Fig. 4.—Case 9.

Fig. 5.—Case 11.   Fig. 12.—Case 34.

Fig. 6.—Case 16.   Fig. 13.—Case 36.

Fig. 7.—Case 18.   Fig. 14.—Case 51.

Fig. 8.—Case 20.   Fig. 15.—Case 57.

PLATE 1
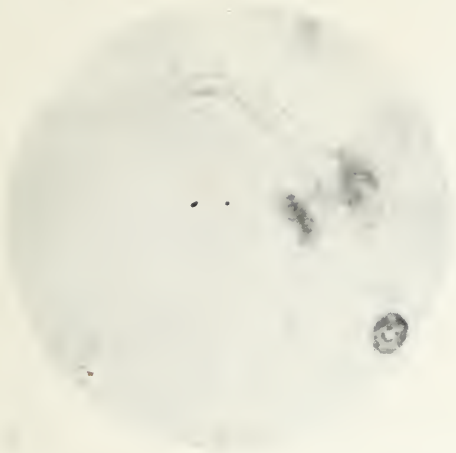
Fig. 1.
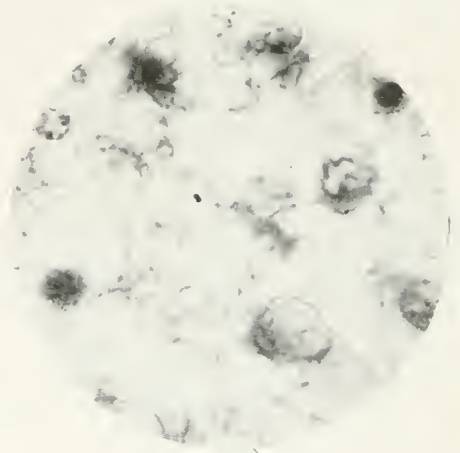
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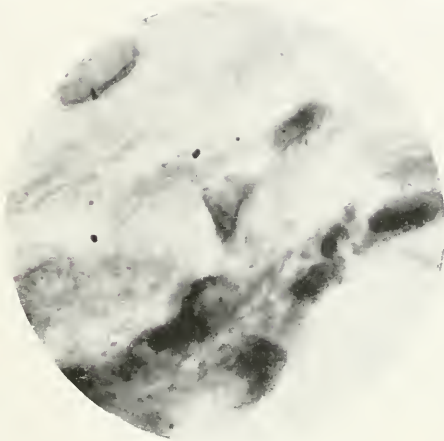
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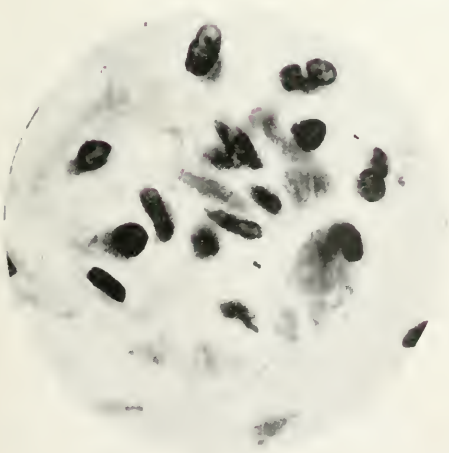
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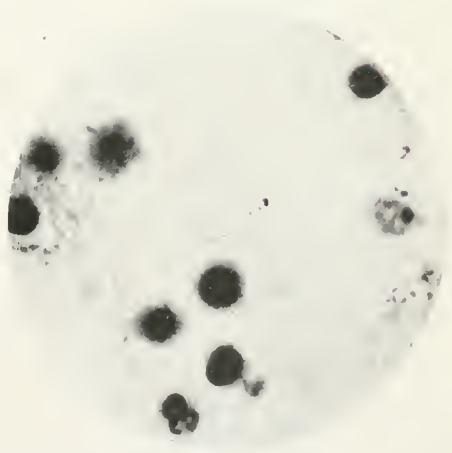
Fig. 5.

PLATE 2
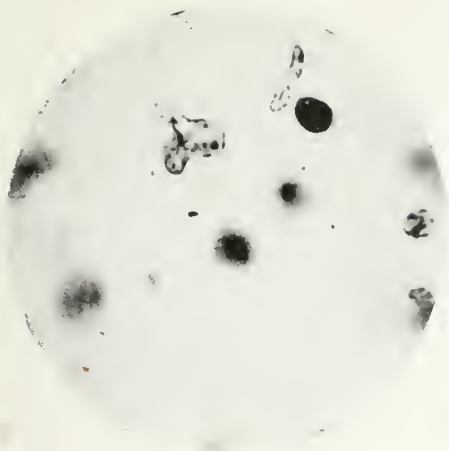
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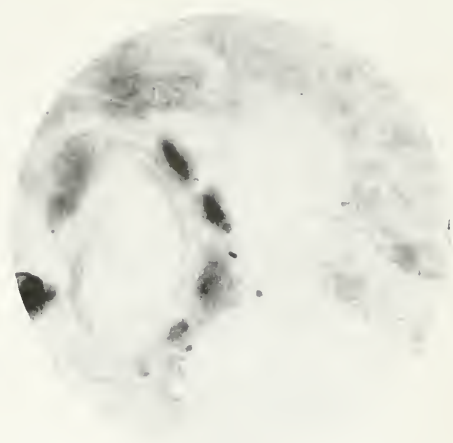
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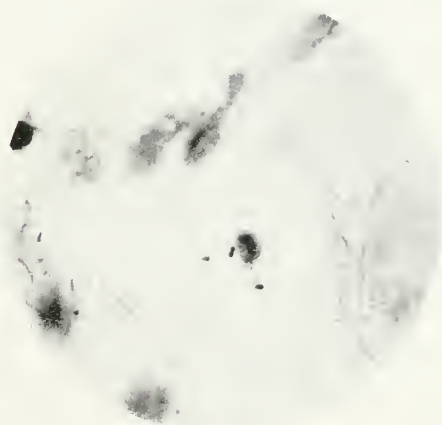
Fig. 8.

Fig. 9.
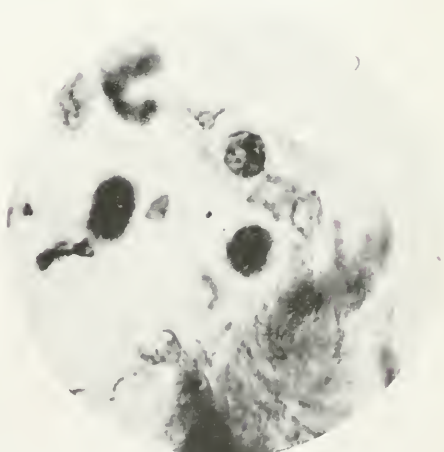
Fig. 10.

PLATE 3
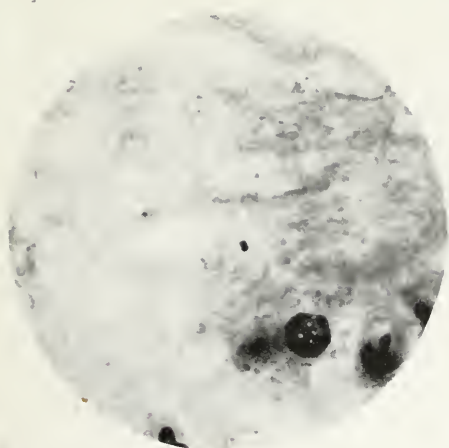
Fig. 11.
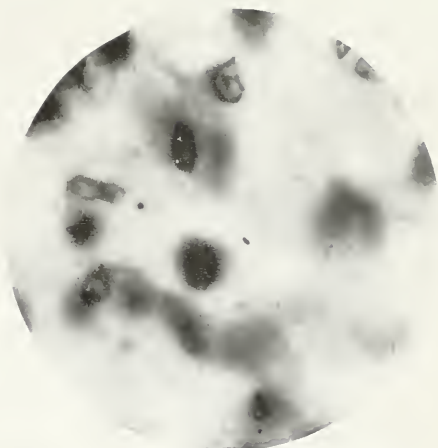
Fig. 12.

Fig. 13.

Fig. 14.

Fig. 15.

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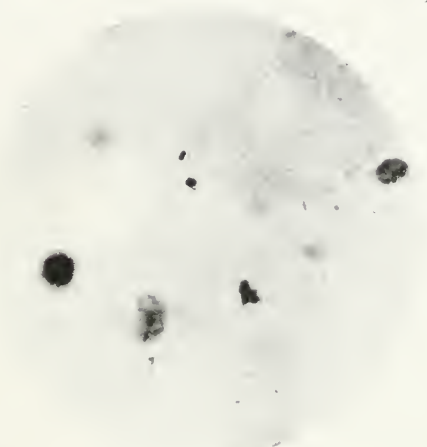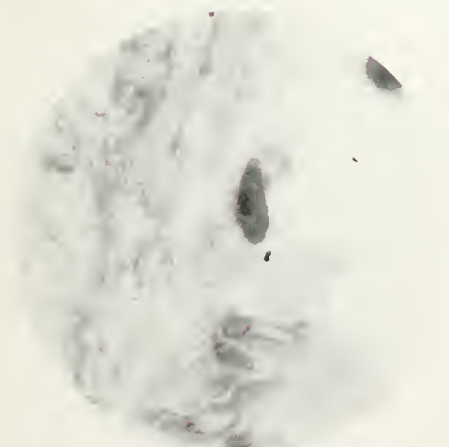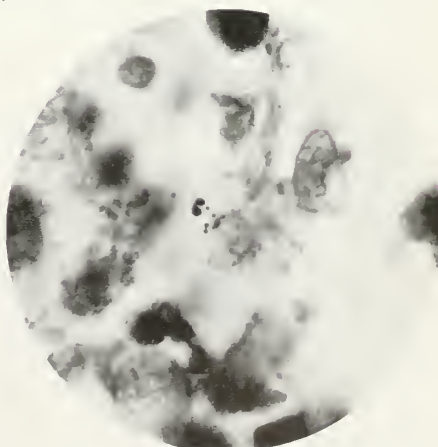
THE INHIBITIVE EFFECT OF OX-BILE ON B. TYPHOSUS

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Bile media have been used extensively, as recommended by Jackson,[1] for rapid isolation of B. coli in sanitary water analysis. They have also been proposed for the isolation of B. typhosus from the blood of patients suffering with typhoid fever (Conradi,[2] Kayser,[3] Fornet[4] and others). That the action of bile on B. coli, however, is inhibitive has been conclusively shown by Jordan[5] and confirmed by Obst.[6]

Since common air and water bacteria and intestinal organisms are for the greater part eliminated through the use of bile mediums, such mediums have certain advantages in the isolation of B. coli and B. typhosus. Bile has even been regarded by some as favoring the growth of these species. Lately, however, experiment tends to show that the effect is inhibitory rather than favorable on typhoid as well as on colon bacilli.

REVIEW OF THE LITERATURE

As far back as 1892, Corrado[7] stated that bile has but a slight effect on the growth of typhoid bacilli; in some instances, the reaction of the bile might have been even slightly inhibitive as stated by that author.

That bile may stimulate the growth of typhoid bacilli was asserted by Fischer,[8] Babes,[9] and Neufeld,[10] while Leubuscher,[11] Talma,[12] and Braun,[13] reported a transitory inhibition. Pies[14] maintains that ox-bile inhibits B. typhosus to a

[1] Biological Studies by the Pupils of William Thompson Sedgwick, Boston, 1906.
[2] Deutsch. med. Wchnschr., 1906, 32, p. 58.
[3] München. med. Wchnschr., 1906, 53, pp. 823, 896, and 1953.
[4] Ibid., 1906, 53, p. 1862.
[5] Jour. Infect. Dis., 1913, 12, p. 326.
[6] Jour. of Bacteriol., 1916, 1, p. 73.
[7] Centralbl. f. Bakteriol., 1892, 12, p. 696.
[8] Ueber die Wirkung der Galle auf Typhus und Milzbrandbazillen, Inaug. Diss., Bonn, 1894.
[9] Berl. klin. Wchnschr., 1899, 17, p. 362.
[10] Ztschr. f. Hyg., 1900, 34, p. 454.
[11] Ztschr. f. klin. Med., 1890, 17, p. 472.
[12] Centralbl. f. Bakteriol., I, 1901, 29, p. 367.
[13] Arch. d. Sci. Biol., 1901, 8, p. 158.
[14] Ueber das Wachstum von Typhus und Colibazillen in Galle, Inaug. Diss., Strassburg, 1907.

more extensive degree than it does B. coli. In his investigations he found one specimen of human bile that inhibited both organisms markedly. According to the same author, the addition of nutrient substances to the bile accelerated the growth of the bacilli, thus neutralizing the inhibitory action of the bile. Dunschmann,[15] who studied the effect of bile and bile salts on B. typhosus and B. coli, reported a favorable action of the bile on B. typhosus and slight inhibition of the growth of B. coli. Hirokawa[16] found that B. coli, B. typhosus and B. paratyphosus A and B develop very well in bile mediums. The author mentions one case of distinct inhibition of typhoid bacilli and states that the power of restraining is slightly or not at all impaired through heat. Fränkel and Krause[17] have reported that human bile stimulates the growth of typhoid bacilli, B. coli, Staphylococcus pyogenes, and Vibrio cholerae. Fornet[18] was able to find but slight difference in the inhibitive effect of steam heated and fresh bile on B. typhosus. When a few typhoid organisms are inoculated into bile, they suffer inhibition to a relatively greater degree according to Fornet. Lange and Roos[19] found that rabbit's bile inhibited typhoid bacilli only to a very slight degree. Tonney, Caldwell and Griffin[20] concluded that "The effect of lactose peptone bile enrichment when used in the attempt to isolate bacilli from urine and feces is that of inhibition of the growth of the bacilli. As a rule, typhoid organisms that are demonstrable by direct plating are lost by passage through lactose bile." Nichols[21] states that rabbit bile in vitro may be antiseptic to B. typhosus, B. paratyphosus A, B. coli and B. dysenteriae. The cholera vibrio which is known to grow best in alkaline culture media, grew well in fresh alkaline bile. The antiseptic action is largely due to its alkalinity. It is apparently possible to protect the rabbit to some degree against gall-bladder infection by a previous injection of sodium carbonate.

From this general survey of the literature it is plain that the results obtained by different workers are more or less at variance.

STRAINS OF TYPHOID BACILLI USED

In our experiments the strains employed were obtained as follows:

1. B. typhosus 1 from the laboratory stock cultures. Source unknown.

2. B. typhosus 2, from Dr. Theobald Smith's laboratory, was isolated on Aug. 28, 1900, from the spleen of a typhoid patient.

3. B. typhosus B was isolated on Dec. 17, 1914, from the urine of a typhoid patient.

4. B. typhosus J was isolated from the blood of a patient on Dec. 15, 1914.

[15] Ann. de l'Inst. Pasteur, 1909, 23, p. 29.
[16] Centralbl. f. Bakteriol., I, O., 1909-1910, 53, p. 12.
[17] Ztschr. f. Hyg., 1899, 32, p. 91.
[18] Arch. f. Hyg., 1907, 60, p. 134.
[19] Arb. a. d. k. Gsndhtsamte, 1915, 57, p. 5.
[20] Jour. Infect. Dis., 1916, 48, p. 239.
[21] Jour. Exper. Med., 1915, 24, p. 497.

5. B. typhosus C was isolated from the stool of a patient on Jan. 16, 1915.

All the suspensions used were made up from broth cultures incubated at 37 C. for 24 hours. In all of the experiments the colony count was made after 48 hours at 37 C.

Experiments 1, 2, and 3

As the addition of other nutrient substances may have a more or less beneficial influence on the different strains, the following mediums were made up in the first 3 series of experiments:

A. Full fresh ox-bile, 1000 c.c., agar, 15 gm.

B. Full fresh ox-bile, 1000 c.c., agar 15 gm., peptone (Difco), 10 gm.

C. Full fresh ox-bile, 1000 c.c., agar, 15 gm., peptone (Difco), 10 gm., and lactose, 10 gm.

D. Full fresh ox-bile, 1000 c.c., agar, 15 gm., peptone (Difco), 10 gm., and glycerol, 10 gm.

The agar, peptone and lactose were dissolved in the bile without addition of water as rapidly as possible by gentle boiling. The medium was filtered and allowed to cool and again boiled and refiltered without titration. It was then tubed and sterilized in the autoclave for 3 minutes at 15 lbs. pressure. The elimination of the precipitate as much as possible during the cooling and refiltering is of great importance for a later count of the colonies. Table 1 shows the inhibitive action of bile on the growth of typhoid bacilli. The better growth obtained in the glycerol series may be attributed to a lessening of the bactericidal effect of the bile or to the favoring of the organisms by the glycerol. Typhoid bacilli are thus restrained through the use of whole fresh ox-bile.

TABLE 1 (UNDILUTED BILE)

Average Results Obtained with Plain Bile and Bile Containing Different Nutrient Substances Compared with Those Obtained with Plain Agar

Strains	Plain Agar	A Bile Agar	B Peptone Bile Agar	C Lactose Peptone Bile Agar	D Glycerol-Peptone Bile Agar
No. 1	224	153	121	70	128
No. 2	122	0	0	1	101
B	290	82	44	89	150
J	175	92	98	124	102
C	209	13	13	20	98

In this series, as recorded in Table 2, 50% bile (diluted with distilled water) was used and the mediums prepared in the same manner as in the first experiment. The comparative results set forth seem to indicate that a great number of the organisms refuse to grow on bile mediums. The glycerol-peptone-bile-agar had a slight inhibitive effect on some of the strains. The colonies on the glycerol-containing medium were much larger and showed a strikingly vigorous growth.

TABLE 2 (50% BILE)

AVERAGE RESULTS OBTAINED WITH THE SAME NUTRIENT SUBSTANCES BUT WITH A DIFFERENT DILUTION OF BILE

Strains	Plain Agar Control	Bile Agar	Peptone Bile Agar	Lactose Peptone Bile Agar	Glycerol Peptone Bile Agar
No. 1	192	120	50	60	200
No. 2	150	73	15	6	60
B	441	73	53	175	400
J	800	500	300	550	430
C	157	7	16	11	63

TABLE 3 (10% BILE)

RESULTS OBTAINED WHEN NUTRIENT SUBSTANCES WERE USED WITH A HIGHLY DILUTED BILIARY SOLUTION

Strains	Plain Agar Control	Bile Agar	Peptone Bile Agar	Lactose Peptone Bile Agar	Glycerol(10%) Pentone Bile Agar
No. 1	600	83	18	582	0
No. 2	212	45	96	187	10
B	543	50	0	470	3
C	386	13	327	300	8

In Exper. 3, 10% bile (diluted with distilled water) was prepared with agar, peptone, lactose and glycerol as in Exper. 1. The results given in this 3rd table are of interest not only because the bile retained its suppressing power in such a high dilution, but also because the inhibitive effect of lactose-peptone-bile-agar here is practically eliminated while the addition of 10% glycerol brings about a uniform inhibition of the cells of all 4 strains. These findings indicate that bile inhibits a large proportion of the otherwise viable cells and the more concentrated the fluid, the greater its effect.

STERILIZATION OF BILE BY FILTRATION

Freshly obtained bile was filtered through a Buchner funnel and passed through a Nordmeyer bougie of 185 by 25 mm., tested for sterility and divided with aseptic precautions in portions of 10 c.c. in test tubes and kept on ice. At this temperature the bile often turns cloudy with the formation of a brown precipitate in the bottom of the tubes, which readily disappears when the fluid is brought to room temperature.

EXPERIMENT 4

The strains were plated with 5 c.c. of the filtered bile which was heated to 45 C. and mixed with 5 c.c. of agar prepared by dissolving 15 gm. of agar in 500 c.c. of tap water. The results were compared with plain agar.

EXPERIMENT 5

A suitable suspension of typhoid bacilli was mixed with plain agar to which 1 c.c. of filtered sterile bile was added after the agar was cooled down to 43 C. Control series were made in plain agar without bile. Again the inhibitive effect is plainly apparent here, but very much weaker. In this case nutrient agar plays an important rôle in furnishing the more nutrient substances favoring the growth of the bacilli.

TABLE 4

SHOWING THE INHIBITIVE EFFECT OF UNHEATED FILTERED BILE

Strains	Plain Agar	Filtered Bile Agar
No. 1	755	7
No. 2	406	3
J	409	10
C	59	7

On this medium B. typhosus is practically unable to grow.

TABLE 5

SHOWING THE INHIBITIVE EFFECT OF FILTERED BILE

Strains	Plain Agar	Bile Agar
No. 1	28	15
No. 2	46	23
B	40	39
J	130	130
C	53	32

EXPERIMENT 6

A loopful of a 24-hour typhoid suspension was added to 10 c.c. of broth and from this suspension after thorough mixing one loop was carefully plated out by washing the loop in 1 c.c. of 0.85% salt solution previously added to the plate. Several plates were made so as to obtain a fair average of the total number of organisms carried by the loop. After this the same procedure was followed except that 10 c.c. of the sterile filtered bile replaced the broth. Table 6 gives the numbers of bacteria surviving exposure to filtered ox-bile for different periods of time. A marked decrease of the otherwise viable cells takes place after the tubes have been shaken. Within the first 5 minutes a great majority are destroyed or their growth prevented. After 20 minutes the increase from the suviving organisms is fairly constant.

TABLE 6

SHOWING THE RAPID INHIBITION OF OTHERWISE VIABLE CELLS

Strains	Controls	Minutes						
		1	5	10	15	20	30	40
No. 1..........	25	18	31	39	20	20	18	28
No. 2	15	1	3	4	4	1	2	3
B.............	57	35	20	32	24	23	35	34
J.............	52	2	1	2	5	5	13	17
C.............	45	22	29	32	35	25	35	40

EXPERIMENT 7

The same experiment was repeated with one strain; the bile used was unheated, filtered in 1 instance, and heated, filtered in the other (3 minutes at 15 lbs. in the autoclave). The results in this experiment indicate that the inhibitive power of filtered bile, when heated for 3 minutes in the autoclave

at 15 lbs., is not destroyed, thus confirming the findings of Fornet and Neufeld. There is a marked inhibition of the organisms after 10 minutes in the non-heated medium, but this may indicate a slighter reaction on the part of the nonheated bile when compared with that of the heated.

TABLE 7

SHOWING THE EFFECTS OF HEATED AND NONHEATED BILE*

	Minutes						
	1	5	10	15	20	30	40
Heated filtered	108	100	112	100	100	98	69
Nonheated filtered..	124	110	50	95	125	118	194

* Strain C: 208 organisms carried by the loop used.

EXPERIMENT 8

In Table 8 the results are given from platings with a suitable suspension of typhoid bacilli in plain agar and the same agar with varying amounts of precipitated mixed bile salts. The mediums were sterilized for 3 minutes in the autoclave at 15 lbs. pressure. A pronounced reduction of the organisms takes place when bile salts are added to nutrient agar. This reduction is constant.

TABLE 8

SHOWING THE INHIBITIVE EFFECT OF BILE SALTS

Strains	Plain Agar Controls	0.05 Per Cent.	Bile-Salts-Agar		
No. 1	387	312	178	241	161
No. 2	920	332	250	226	170
B	1,020	876	900	626	480
J	23	20	16	11	0
C	960	870	840	636	354

SUMMARY

1. The recorded observations show that a great number of the viable cells of B. typhosus are affected through the use of bile media, and further multiplication prevented. Bile has a distinct inhibitive effect on the growth of typhoid bacilli.

2. This inhibition can be reduced to a certain extent by the addition of glycerol.

3. There is no marked difference between the inhibitory power of bile sterilized by heat and that of bile sterilized by filtration through a Nordmeyer bougie.

4 Bile salts distinctly retard the growth of typhoid bacilli.

A CONTRIBUTION TO THE THEORY OF ANAPHYLACTIC SHOCK *

J. L. KRITCHEWSKY

PLATE 4.

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Doerr and Russ,[1] and Doerr and Moldovan[2] demonstrated that there exists a parallelism between the amount of precipitins in immune serum and its power to provoke the state of passive anaphylaxy in normal animals. Friedemann[3] observed the same dependence between this power and the content of hemolysins in the serum. Scott[4] proved that the intensity of the anaphylactic shock is proportionate (in a sensitized animal) to the content of precipitins in its serum, these precipitins being consumed during the shock. Though one cannot affirm with certitude the identity of the precipitins and hemolysins on the one hand, and the antibodies provoking the anaphylactic shock on the other, yet these data, confirmed by other workers, show that the parallelism of their existence is an undisputable fact.

In several articles[5] I have presented the results of my observations and experiments concerning the proprieties of the juice of a plant, Cotyledon scheideckeri, which is able to precipitate animal serum and bacterial extracts, to agglutinate bacteria and blood corpuscles, and to lake the latter. Now, with a view to the facts stated by Friedemann, Doerr and Russ, Doerr and Moldovan, and Scott, I undertook to solve the problem, whether the cotyledon juice, possessing such an influence over serum and animal cells, has the power to provoke the clinical and anatomic changes characteristic of anaphylactic shock, when introduced into the living animal. This is the object of the present investigation. The experiments gave a positive answer. The guinea-pigs and rabbits which received the fluid intravenously died; the clinical picture and the anatomic changes were those of anaphylaxis.

The clinical phenomena are generally as follows: The guinea-pig after being taken from the table (this requires 15-25 seconds) remains

[1] Ztschr. f. Immunitätsforsch., 1909, 3, p. 706.
[2] Ibid., 1910, 2, p. 161.
[3] Ibid., 1909, 2, p. 591.
[4] Jour. Path. and Bacteriol., 1910, 14, p. 147.
[5] Ztschr. f. Immunitätsforsch., 1914, 22, p. 381; 1915, 23, p. 321.

very quiet for some time, generally 15-45 seconds; only now and then it has a brief chill; then begin tonic and clonic convulsions of the muscles of the extremities, the trunk and the neck, during which the animal falls on its side. The period of convulsions is followed by dyspnea, lasting a longer time than the preceding phase and ending with death in a comatose state. The intervals between the respiratory movements grow each time longer; finally, the act of breathing becomes a catching and swallowing of the air. The reflexes of the cornea gradually disappear. In this period urine is often voided. Sometimes convulsive wormlike movements of the paws are observed. In some cases a foaming liquid tinged with blood comes from the mouth and nostrils the moment before death.

Rabbits offer a similar picture. An intense opisthotonus is frequently observed; sometimes the head is turned up almost at a right angle to the body, and in this position the animal dies. Often the rabbit gives a scream during the dyspnea. Now and then the dyspnea does not appear at all; but exophthalmus is always observed.

EXPERIMENT 1

Only the facts which vary from one experiment to another are mentioned in the protocols.

GUINEA-PIGS

1. Weight, 340 gm.; 10 c.c. Cotyledon Fluid 5 into jugular vein; died in 2 minutes; lungs greatly inflated, emphysematous; no thrombi.

2. Weight, 340 gm.; 8.5 c.c. Fluid 5 into jugular vein; death in 3 minutes; lungs greatly distended, punctate hemorrhages; thrombus in right heart.

3. Weight, 340 gm.; 5 c.c. Fuid 5 into jugular vein; no effect.

4. Weight, 310 gm.; 6 c.c. Fluid 8 into jugular vein; death in 4½ minutes; lungs greatly inflated; thrombus in right heart.

5. Weight, 305 gm.; 4 c.c. Fluid 8 into jugular vein; died in 3 minutes; strongest lung inflation; thrombus in right and left heart.

6. Weight, 310 gm.; 2 c.c. Fluid 8 into jugular vein; no effect.

7. Weight, 370 gm.; 10 c.c. Fluid 10 into jugular vein; death in 3 minutes; moderate lung inflation; no thrombosis in heart cavities; thrombus in inferior vena cava.

8. Weight, 375 gm.; 6 c.c. Fluid 10 into jugular vein; death in 2½ minutes; strong lung inflation; thrombus in right heart.

9. Weight, 375 gm.; 4 c.c. Fluid 10 into jugular vein; fell on side and convulsions; after 1½ minutes it recovered and arose; 5½ minutes after injection it fell again; convulsions, dyspnea, and death in 2 minutes. No thrombus in heart cavities nor lungs; no lung inflation.

10. Weight, 380 gm.; 2 c.c. of Fluid 10 into jugular vein; no effect.

11. Weight, 405 gm.; 4 c.c. Fluid 9 into jugular vein; no effect during first 3 minutes; then convulsions; yet after 2 minutes the animal recovered.

12. Weight, 360 gm.; 6 c.c. Fluid 9 into jugular vein. Death in 2 minutes; strong lung inflation; thrombus in right heart.

13. Weight, 360 gm.; 4 c.c. Fluid 12 into jugular vein immediately after taking 4 c.c. of blood from the heart. Death followed in 3 minutes; no lung inflation; the thorax was full of blood and clots; no thrombus in the heart cavities.

14. Weight, 355 gm.; 6 c.c. of Fluid 12 into jugular vein immediately after 4 c.c. of blood were taken from the heart; death after 2½ minutes; slight lung inflation; no thrombus in the heart.

15. Weight, 355 gm.; 4 c.c. of Fluid 14 into jugular vein; death after 2 minutes; strong lung inflation; thrombus in right heart; before the experiment 4 c.c. of blood were taken from the heart.

16. Weight, 515 gm.; 6 c.c. Fluid 14 into jugular vein. No effect during 15 minutes; then began convulsions; the pig fell on its side; after the short convulsive period there followed dyspnea and a deep comatose state, which lasted 55 minutes; death after 71 minutes from the beginning of the experiment; no lung inflation; no thrombus in the heart.

17. Weight, 470 gm.; 4 c.c. Fluid 16 into jugular vein; convulsions, fell on the side; yet after 25 seconds it arose; recovered.

18. Weight, 410 gm.; 8 c.c. Fluid 16 into jugular vein; convulsions; fell on the side; dyspnea; after 1 minute it arose; recovered.

19. Weight, 455 gm.; 8 c.c. Fluid 15 into jugular vein; death in 7½ minutes; slight lung inflation; thrombus in right heart.

20. Weight, 360 gm.; 4 c.c. Fluid 21 into jugular vein. Death in 2½ minutes; strong lung inflation; thrombus in right heart.

21. Weight, 455 gm.; 4 c.c. of Fluid 22 into jugular vein; death in 2½ minutes; strong lung inflation and punctate hemorrhages under the pleura; thrombus in right heart. Before the injection 4 c.c. of blood were taken from the heart.

22. Weight, 386 gm.; 4 c.c. Fluid 23 into jugular vein; no effect during 5 minutes; then convulsions, dyspnea, and coma; then convulsions recommenced and were again followed by a deep comatous state which lasted 1 hour and 40 minutes. The pig died 2 hours and 10 minutes after the beginning of the experiment. Strong lung inflation; lung hemorrhages; no thrombus in heart. Punctate hemorrhages in the mucous membrane of stomach and intestines.

23. Weight, 385 gm.; 4 c.c. Fluid 25 into jugular vein; death in 2 minutes; slight lung inflation and punctate hemorrhages; thrombus in right heart. Before the injection 4 c.c. of blood were taken from the heart.

24. Weight, 405 gm.; 4 c.c. Fluid 25 into jugular vein; death in 2 minutes; no lung inflation; punctate hemorrhages and intense hyperemia; no thrombus in heart. Before the injection 4 c.c. of blood were taken from the heart.

25. Weight, 335 gm.; 4 c.c. Fluid 29 into jugular vein; death after 2 minutes; strong lung inflation; thrombus in right heart.

26. Weight, 340 gm.; 4 c.c. Fluid 28 into jugular vein; death after 2 minutes; strong lung inflation and capillary hemorrhages; thrombus in right heart.

27. Weight, 300 gm.; 4 c.c. Fluid 30 into jugular vein; death after 2 minutes and 15 seconds; slight lung inflation; thrombus in right heart.

28. Weight, 335 gm.; 4 c.c. Fluid 32 into jugular vein; death in 2 minutes; strong lung inflation; thrombus in right heart.

29. Weight, 330 gm.; 5 c.c. Fluid 29 into jugular vein; death after 25 minutes; strong lung inflation; no thrombus in heart.

1. Weight, 560 gm.; received 10 c.c. of Fluid 1 in ear vein; death followed after 2 minutes. No thrombus in the heart.

2. Weight, 670 gm.; received 5 c.c. of Fluid 1 in the ear vein; convulsions, from which the animal recovered very soon; after 45 minutes convulsions again for 9 minutes; dyspnea and death; thrombus in the right heart.

3. Weight, 560 gm.; received 5 c.c. of Fluid 1 in ear vein; death after 3 minutes; thrombus in the right heart.

4. Weight, 570 gm.; received 10 c.c. of Fluid 12 in the ear vein with no effect.

5. Weight, 495 gm.; received 10 c.c. of Fluid 6 in the jugular vein; death after 1½ minutes; thrombus in the right and the left heart.

6. Weight, 740 gm.; received 10 c.c. of Fluid 7 in the ear vein, with no effect.

7. Weight, 570 gm.; received 10 c.c. of Fluid 12 in the ear vein; death after 1½ minutes; no thrombus in the heart.

8. Weight, 490 gm.; received 4 c.c. Fluid 14 into the ear vein; death after 1½ minutes; thrombus in the right heart.

9. Weight, 590 gm.; received 4 c.c. of Fluid 25 in the ear vein; death after 5 minutes; thrombus in the right heart.

The necropsy of the guinea-pigs revealed the following picture: For the most part the lungs were so inflated that they filled the thorax entirely and covered the greatest part of the heart. They were immobile and did not collapse. A slight inflation or the complete absence of inflation was rather rare. In the majority of cases the lungs were hyperemic and of pale pink color. The hemorrhages, seen macroscopically, were not frequent; generally they were punctate. Sometimes I found in the emphysematous tissue slightly shrunken parts of normal not inflated lungs.

The microscopic examination revealed a marked dilatation of the alveoli (Fig. 1); the walls of the alveoli were frequently torn; the vessels and capillaries were for the most part in a state of intense hyperemia; in some cases the extension of the capillaries went so far that they looked like big spherical formations (Fig. 2) filled with real corpuscles; in this state ruptures of the walls occur.

The capillary hemorrhages seldom involved large parts of the lung tissue; when they did, this was from diapedesis and not because of excessive extension (Fig. 4). The contraction of the bronchial openings and the plaits and folds in the mucous membrane of the bronchi were present in the majority of cases, yet not always (Fig. 3).

In the right heart, and the vessels leading to it, thrombosis was frequent; now and then in the left heart also; sometimes there was

none at all. If the necropsy was made immediately after death the heart was nearly always still beating, sometimes as long as 11 minutes. When there was thrombi in the heart, they appeared now and then also in the vena cava inferior.

The retardation of the coagulation of the blood was insignificant. The organs of the abdomen were full of blood, also the vessels of the mesentery; the gallbladder was frequently distended. In some cases capillary hemorrhages were observed in the mucous membrane of the stomach and the intestine.

As to the rabbits: their lungs were not inflated; for the most part the lungs were hyperemic. In the heart, thrombi were generally present, yet not always; often also in the inferior cava. The blood of the rabbits, contrarily to that of guinea-pigs, did not coagulate for a very long time, sometimes not for 24-36 hours. The organs of the abdomen were full of blood; the liver especially.

My experiments show that thrombosis was very frequent; in anaphylaxis it is said to be rarely found. Two reasons, however, do not allow a sharp demarcation between anaphylactic shock and the shock I observed. First of all, the formation of thrombus in the heart during life can be observed even in the conditions of a real anaphylactic experiment, if one injects into a fresh animal the immune serum against its own albumin, instead of injecting antigen into sensitized animals. Besides, the protocols show that thrombi were not present in all cases; so one cannot attribute the death to thrombosis of the heart and the vessels, moreover, the clinical phenomena are identic when thrombosis occurs and when it does not occur. Inflation and immobility of the lungs in connection with a specific clinical picture being considered as important signs of anaphylactic shock, I would emphasize that the condition of the lungs in my experiments entirely completes the series of analogies with anaphylactic shock. I mentioned that sometimes I did not discover any folds in the mucous membrane of the bronchi nor any contraction of the bronchial openings, but this may be the case even in anaphylaxis (Kumagai[6]).

Thus, on basis of the preceding argument, one may draw the conclusion that the clinical phenomena and the anatomic changes which accompany the shock caused by the injection of cotyledon fluid into the vein are those of the anaphylactic shock. From this one can infer that in order to provoke the clinical picture and the anatomic changes

[6] Ztschr. f. Immunitätsforsch., 1913, 17, p. 602.

characteristic of anaphylactic shock, it is necessary to create in the animal organism the conditions which are indispensable in order to obtain precipitation in serum or lysis and agglutination of red corpuscles in vitro; and it is quite indifferent whether this effect is due to the previous immunization of the animal, which is the object of the experiment or which furnishes the immune serum, or to some substance quite independent of the animal the object of the experiment.

EXPERIMENT 2

The experiments now to be described were undertaken in order to study the relation between the precipitating and agglutinating powers of the cotyledon fluid on the one hand, and its power to provoke the clinical picture of the anaphylactic shock on the other. It appeared plausible a priori that the fluid when deprived of all precipitating and agglutinating substances would be quite harmless for animals. The experiments fully confirmed this supposition. The procedure was as follows: First of all, I determined the fatal dose of the fluid of the plant; this quantity was mixed with a definite quantity of serum or bacterial mass which was directly washed off with the fluid from agar slants; after the development of precipitation or agglutination the sediment was separated from the liquid by means of centrifugation. The liquid was tested in order to ascertain whether it was completely devoid of precipitating and agglutinating substances, or not; finally, it was injected into the jugular vein in quantity equal to the fatal dose, or slightly surpassing it.

GUINEA-PIGS

All injections were made into the jugular vein.

1. Weight, 305 gm.; received 5 c.c. of fluid prepared with 10 c.c. of Cotyledon Fluid 8 + 2 c.c. of horse serum; no effect.

2. Weight, 310 gm.; received 5 c.c. of fluid made with 10 c.c. of Fluid 8 + 2 c.c. of horse serum; no effect.

3. Weight, 315 gm.; received 4.5 c.c. of fluid made with 7.5 c.c. of Fluid 8 + 2 c.c. of agar cultures of B. coli; no effect.

4. Weight, 345 gm.; received 6.5 c.c. of fluid made of 8 c.c. of Fluid 12 + 1 c.c. of horse serum; no effect.

5. Weight, 345 gm.; received 6 c.c. of fluid made of 8 c.c. of Fluid 12 + 3 cultures of B. coli; death followed after 1½ minutes; strong lung inflation; thrombus in the right heart.

6. Weight, 270 gm.; received 6 c.c. of fluid of 7.5 c.c. of Fluid 9 + 2 cultures of B. coli; no effect.

7. Weight, 370 gm.; received 5 c.c. of fluid made of 10 c.c. of Fluid 14 + 2 c.c. of horse serum; no effect during one minute; then followed the usual picture of shock. At the end of the deep comatose state a bloody foam came

from mouth and nostrils. The lung inflation was insignificant; many hemorrhages in the lungs; edema; no thrombus in the heart; death after 3½ minutes.

8. Weight, 290 gm.; received 10.8 c.c. of fluid composed of 10 c.c. of Fluid 14 + 8 c.c. of horse serum. Brief convulsions; after 20 minutes the animal was quite recovered.

9. Weight, 345 gm.; received 10 c.c. of mixture of 10 c.c. of Fluid 15 + 1 c.c. of horse serum; convulsions and dyspnea; then slow recovery.

10. Weight, 300 gm.; received 10 c.c. of Fluid 15 treated with 4 tubes of B. coli slant agar. Usual phenomena and death after 3 minutes; strong lung inflation; thrombus in the right heart.

11. Weight, 310 gm.; received 8 c.c. of fluid composed of 10 c.c. of Fluid 14 +. 1 c.c. of horse serum; usual picture; a bloody foam came from mouth and nostrils; intense lung inflation; hemorrhages; edema; death after 4 minutes.

12. Weight, 370 gm.; received 5 c.c. of fluid composed of 10 c.c. of Fluid 21 + 3 c.c. of horse serum; no result during 8 minutes; then the usual phenomena of shock; death after 7 minutes. Intense lung inflation; several hemorrhages under the pleura; thrombus in the right heart.

13. Weight, 310 gm.; received 8 c.c. of fluid composed of 10 c.c. of Fluid 21 + 10 c.c. of horse serum; no effect.

14. Weight, 450 gm.; received 4.8 c.c. of fluid composed of 5 c.c. of Fluid 22 + 1 c.c. of horse serum; no effect.

15. Weight, 360 gm.; received 4 c.c. of Fluid 25 + 3 c.c. of horse serum; no effect.

16. Weight, 320 gm.; received 5 c.c. of fluid composed of 4 c.c. of Fluid 29 + 7 c.c. of horse serum; no effect.

17. Weight, 320 gm.; received 4 c.c. of fluid composed of 11 c.c. of Fluid 29 + 10 agar cultures of B. coli; no effect.

18. Weight, 300 gm.; received 4.5 c.c. of fluid of 10 c.c. of Fluid 30 + 10 agar cultures of B. coli; no effect.

19. Weight, 305 gm.; received 4.5 c.c. of Fluid 32 + 8 cultures of B. coli; no effect.

Thus, in the majority of cases the disappearance of the precipitating and agglutinating substances deprived the cotyledon fluid of the power to cause shock in the animal. In 5 animals only was shock and death provoked, in spite of the precipitating and agglutinating substances having been removed from the cotyledon fluid. These cases sometimes offered the picture of lung edema and of diffuse hemorrhages, revealed by the foaming bloody liquid which came from mouth and nostrils. Lung edema is observed also in anaphylaxis (Kumagai[6]). I must try to give an explanation of these cases.

Studying the precipitating action of the cotyledon fluid I discovered a phenomenon in some degree analogous with those observed by Danysz, Bordet and v. Dnngern in their experiments of neutralization of toxins by antitoxins. If 4 c.c. of cotyledon fluid were mixed at once with 1 c.c. of horse serum (samples 22, 23, 24 and 25) and sep-

arated from the sediment by means of centrifugation, further additions
of serum did not provoke any new precipitation; if only 1 c.c. of fluid
was taken, and the serum was added by drops, the quantities of serum
causing precipitation were the following: for Fluid 22—0.5 c.c., 23—
0.8 c.c., 24—0.6 c.c., and 25—0.5 c.c. It results from this that 1 c.c.
of horse serum was insufficient for the complete extraction of all the
precipitating substances from 4 c.c. of fluid; 2.0, 3.2, 2.4 and 4.0 c.c.
of serum were necessary for that purpose. Thus, to speak exactly, in
the majority of my experiments I did not deprive the fluid of all its
precipitating substances; a part of them was left, and though this
remaining part was unable to generate any further precipitation in
vitro, in vivo it might have conserved the power to change the degree
of the disperseness of the plasma colloids and to cause shock and death
in this way. In fact, the fluid treated with large quantities of serum
had no action on the animals, while the same fluid treated with the
quantities that I generally used in the experiments killed the guinea-
pigs (refer to experiments with Fluids 14 and 21). It is probable that
all this may be affirmed also of the agglutinating substances of the
cotyledon fluid (note experiments with Fluids 12, 15, 30 and 32).

Referring to parallelism between the action of the fluid in vitro
and in vivo, I have not the intention to affirm that the fluid provokes
in the animal agglutination of blood corpuscles, or precipitation in
the plasma similar to that in the serum. Yet, the indispensableness of
precipitating substances in the fluid for the production of shock sug-
gests the idea that in the blood system of animals the fluid changes
always the degree of the disperseness of the plasma colloids, and thus
causes shock and death. In vitro the change of the disperseness of
the serum colloids always causes the visible formation of gel under
the form of precipitate; in the animal the formation of gel seen with
the eye under the form of thrombus is not always observed.

On the basis of the results given one may conclude that the coty-
ledon fluid provokes a shock, which offers a clinical picture and ana-
tomic changes analogous to those of anaphylactic shock; the shock and
the death of the animal are due to change in the degree of disperseness
of the plasma colloids, and it is indifferent whether this change causes
the formation of thrombus or not.

EXPERIMENT 3

The experiments now described corroborate the supposition that
the power to provoke the shock and death of animals of the active

substance of the fluid is due to a physicochemical influence over the blood circulating in the vessels, and not to a chemical injury of some cells of the organism. The fluid injected into the abdomen of guinea-pigs did not affect them at all; neither did a subcutaneous injection.

I studied the chemical nature of the active substance of the coty-ledon together with J. D. Sartakoff, but the circumstances of the war did not allow us to finish that work. This may be stated: the sub-stances of the fluid which cause the agglutination of the bacteria and the red blood corpuscles, the precipitation of serum, the hemolysis of blood corpuscles, the shock and death of animals, are either glucosids, or they pass during precipitation into the sediment together with glu-cosids, because of the great absorbing faculty of the latter. Under the action of a weak soda solution the active substances of the cotyledon fluid pass into the precipitate; this precipitate being again dissolved in a weak solution of acetic acid, the active substances do not lose their various powers.

Guinea-Pigs

1. Weight, 325 gm.; received 10 c.c. of Fluid 5 into the abdomen with no effect.

2. Weight, 300 gm.; received 6 c.c. of Fluid 22 into the abdomen with no effect. On the 3rd day it died of purulent peritonitis.

3. Weight, 350 gm.; received 5 c.c. of Fluid 25 into the abdomen; no effect.

4. Weight, 460 gm.; received 10 c.c. of Fluid 24 into the abdomen with no effect.

EXPERIMENT 4

Not only were the symptoms and the anatomic changes of the shock observed in my experiments those of anaphylaxis as shown by Experiment 4, but the injection of the cotyledon fluid under the skin of animals provokes the condition known as Arthus' phenomenon, ordinarily considered as anaphylactic and to presuppose an earlier sen-sitization of the animal and a subsequent injection of antigen.

Guinea-Pigs

1. Weight. 295 gm.; 4 c.c. of Fluid 22 under the skin of the abdomen; after 24 hours the place of the injection showed edema; after 48 hours the infiltrate grew hard and painful; after 144 hours a placque was formed with ulceration at the margins; after some time this became covered with a crust and finally cicatrized.

2. Weight, 310 gm.; 5 c.c. of Fluid 25 under the skin; edema after 24 hours; after 48 hours a hard infiltrate, which grew smaller after 72 hours and slowly disappeared during the following days.

3. Weight, 385 gm.; 5.25 c.c. of Fluid 30 under the skin of the abdomen; edema after 24 hours; after 48 hours a firm infiltrate, which became doughy

after 96 hours; on pressure it burst open and discharged bloody material; after 144 hours the infiltrate was hard, 6 by 4 cm.; later it was covered with a crust and cicatrized.

4. Weight, 385 gm.; 5 c.c. of Fluid 32 under the skin of the abdomen; there was almost the same picture as in the preceding animals.

RABBITS

1. Weight, 485 gm.; received 4 c.c. of Fluid 29 under the skin of the abdomen; after 24 hours—edema, which increased after 48 hours and grew hard; during the following days it increased still more and attained the firmness of cartilage; its surface became mortified after 288 hours and formed a crust covering a hard mass, which cicatrized afterwards; the scar was starlike.

2. Weight, 560 gm.; received 4 c.c. of Fluid 29 under the skin of the abdomen; there was nearly the same picture as in the preceding case.

3. Weight, 490 gm.; 5 c.c. of Fluid 28 under the skin of the abdomen; edema after 24 hours; after 48 hours a hard infiltrate; its center became mortified after 72 hours; after 144 hours there was a large crust covering a hard area, which cicatrized afterwards.

DIMINUTION OF COMPLEMENT

It is known that Friedberger[7] and workers in his laboratory attach much value to the diminution of the quantity of complement in the state of anaphylaxis, and this fact is cited to confirm their theory of anaphylactic shock being a consequence of the intoxication of the organism with the products of the parenteral digestion of the antigen by the antibodies, with participation of the complement; the latter is partially consumed during the process. The investigations of Busson and Takahashi[8] proved that sometimes there is not observed any decrease of the quantity of complement in the state of anaphylaxis. In my experiments I discovered a diminution of the quantity of complement nearly always during the shock. The complement titer was determined just before the beginning of the experiment, the blood being taken from the heart and immediately after the death. The results of the titration are shown in Table 1. The total quantity in each tube was 2.5 c.c.; 0.5 c.c. of a 5 % suspension of blood corpuscles, and a triple hemolytic dose of a serum with a titer of 1 : 1200 (0.5 c.c.); incubation 30 minutes at 37 C.

In the condition of my experiments the presence of antibodies was excluded; therefore, I believe that the cause of the diminution of the quantity of complement during the shock (and probably during anaphylaxis too) is to be explained in some other way than that of Friedberger. In my opinion, it is more plausible that the complement might

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[7] Ztschr. f. Immunitätsforsch., 1909, 3, p. 581.
[8] Zentralbl. f. Bacteriol., O., I, 1912, 65, p. 146.

be absorbed from the plasma by some substances of the plasma, which show a decrease of their degree of disperseness.

TABLE 1

DIMINUTION IN COMPLEMENT AFTER SHOCK

Experiment	Guinea-Pigs	When Complement Was Obtained	Hemolytic Power of the Guinea-Pig Complement				
			0.01 C.c. of Amboceptor	0.03 C.c. of Amboceptor	0.05 C.c. of Amboceptor	0.07 C.c. of Amboceptor	0.09 C.c. of Amboceptor
1	13	Before the experiment	Slight hemolysis	Incomplete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
		After the experiment	No hemolysis	No hemolysis	Incomplete hemolysis	Incomplete hemolysis	Incomplete hemolysis
1	14	Before the experiment	Slight hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
		After the experiment	No hemolysis	Slight hemolysis	Almost incomplete hemolysis	Complete hemolysis	Complete hemolysis
1	15	Before the experiment	Incomplete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
		After the experiment	No hemolysis	Almost complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
1	21	Before the experiment	Slight hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
		After the experiment	No hemolysis	Incomplete hemolysis	Almost complete hemolysis	Almost complete hemolysis	Almose complete hemolysis
1	23	Before the experiment	Incomplete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
		After the experiment	No hemolysis	No hemolysis	Almost complete hemolysis	Almost complete hemolysis	Almost complete hemolysis
1	24	Before the experiment	Slight hemolysis	Almost complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
		After the experiment	No hemolysis	Slight hemolysis	Incomplete hemolysis	Almost complete hemolysis	Almost complete hemolysis
	92*	Before the experiment	Slight hemolysis	Incomplete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
		After the experiment	Slight hemolysis	Slight hemolysis	Slight hemolysis	Slight hemolysis	Slight hemolysis
	92*	Before the experiment	Slight hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis	Complete hemolysis
		After the experiment	No hemolysis	Incomplete hemolysis	Incomplete hemolysis	Incomplete hemolysis	Incomplete hemolysis

* Not mentioned in Exper. 1.

DISCUSSION

I believe that the data given prove with sufficient probability that the substances provoking the change of the degree of disperseness in

a serum or a suspension of bacterial or animal cells in vitro have the same effect in an animal body, when directly introduced into the blood system, and the result is a shock exactly reproducing the anaphylactic, clinically and anatomically.

My experiments fully corroborate the data of Doerr and Moldovan[9] who obtained a shock similar to the anaphylactic by injecting a water colloidal solution of silicic acid, also of nucleinic acid, and of dialyzed iron hydroxid.

The anaphylactic shock supposes the previous immunization of the animal, and the immune bodies cause the same change in the degree of disperseness in vitro as substances of the cotyledon fluid which I used; it is, therefore, quite natural to suppose that the shock observed in anaphylaxis and related processes just as the shock studied in the present investigation, is due to change in the degree of disperseness of the plasma colloids of the blood. On this account one may qualify all these processes — and others showing an analogous, clinical and anatomic picture — as morbid processes caused by change of the degree of disperseness of the blood plasma. These processes may be grouped as follows:

A. In an immunized organism—

1. Active anaphylaxis.

B. In a not immunized organism, caused by immune serum—

I.—1. Passive anaphylaxis.

2. Shock due to serum against the albumin or the red corpuscles of the animal submitted to experiment.

3. Shock due to hemolytic sheep serum in animals whose organs contain heterogeneous sheep antigen.

4. Shock due to a mixture of antigen and antibody.

II.—Caused by substances of animal origin—

1. Shock due to some normal serum (ox).

2. Shock after the injection of fresh defibrinated blood and of the serum of that blood, or not coagulated blood collected into a paraffined vessel.

3. Shock after the injection of an extract of animal organs, or of the suspension of these organs in physiologic salt solution.

4. Shock after the injection of urine.

[9] Bioch. Ztschr., 1912, 41, p. 27.

5. Shock after the injection of fresh serum treated during some time with bacteria, animal cells, agar, kaolin, etc. (anaphylatoxin).

III.—Caused by substances of unknown chemical origin or by chemical substances—

1. Shock due to substances found in Cotyledon scheideckeri.

2. Shock due to water colloidal solution of silicic acid, nucleinic acid, or dialyzed iron.

This classification has no claim to completeness.

CONCLUSIONS

The sap of a plant, Cotyledon scheideckeri, which is able to precipitate animal serum, and to agglutinate and cause lysis of red blood corpuscles, has the power to provoke the symptoms and the anatomic changes characteristic for anaphylactic shock, when introduced into the veins of animals.

The injection of the cotyledon fluid under the skin of animals provokes phenomena identical with the local anaphylaxis, known as Arthus' phenomenon.

When deprived of all precipitating and agglutinating substances, the fluid is quite harmless for animals.

The shock and the death after injection of the cotyledon fluid are due to change in the degree of the disperseness of the plasma colloids.

In anaphylactic shock in immunized animals immune bodies cause the same change in the degree of disperseness in vitro substances in the cotyledon juice which I examined; it is, therefore, quite natural to suppose that the shock observed in anaphylaxis and related processes just as the shock studied in the present investigations are due to change in the degree of disperseness of the plasma colloids of the blood. On this account one may regard all these processes — and others, showing analogous, clinical and anatomic changes — as morbid processes caused by a change of degree of disperseness of the blood plasma.

EXPLANATION   OF   PLATE   4

FIGS. 1 AND 2.   The alveoli are distended; some of them have torn walls; the capillaries are in a state of strong hyperemia.   Exper. 1, Guinea-Pig 20.

FIG. 3.   The alveoli are distended; some of them have torn walls.   The opening of one bronchus is narrowed; the other bronchus does not possess smooth muscles, and therefore is not contracted.   Exper. 1, Guinea-Pig 25.

FIG. 4.   The alveoli are distended; some of the walls are torn.   Hemorrhages from capillaries per diapedesin.   Exper. 1, Guinea-Pig 21.

PLATE 4
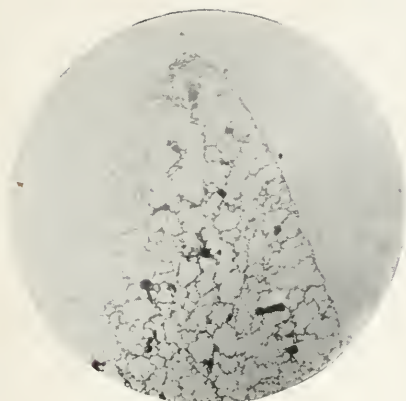
FIG. 1.
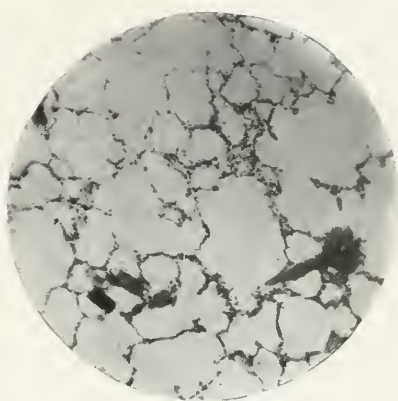
FIG. 2.
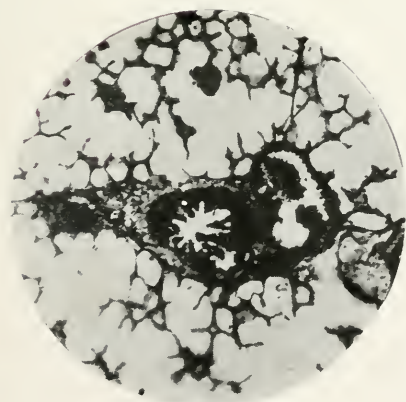
FIG. 3.
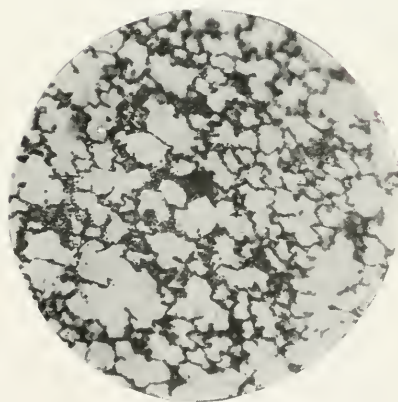
FIG. 4.

SPECIFIC PRECIPITINS IN GONOCOCCAL INFECTIONS

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Since the discovery by Kraus,[1] in 1897, of specific precipitins in anticholera and other immune serum, and of Bordet[2] and Tchistowitch,[3] in 1899, of specific precipitins in the serums of animals injected with foreign proteins, the study of specific precipitation has been pursued extensively. Bruckner and Cristeanu[4] were the first to study precipitins in gonococcal infection, in 1906.

Gonococci grown on blood agar were treated with 0.15% caustic soda, filtered through porcelain, and mixed with antigonococcus serum, in which a marked precipitate was found whereas there was no precipitate in mixtures with normal serum.

Wollstein[5] obtained precipitate with antigonococcus rabbit serum and various forms of extracts of gonococci.

Torrey,[6] using a culture filtrate, obtained marked precipitate with immune rabbit serum, but only a slight reaction with the serum of animals injected with meningococcus, the micrococcus catarrhalis, and none at all with antistaphylococcus serum. He also observed that stronger reactions were obtained with the homologous than the heterologous strains, and this led him to suggest that there may be different types of gonococci, especially as experiments with other immunity reactions indicated that such would be the case.

I have studied the questions of precipitins in gonococcal infection, and in antigonococcus serum, also whether precipitation will distinguish between the gonococcus and the meningococcus, and whether it will permit the grouping of gonococci.

EXPERIMENTS

Eight strains of gonococci were used, the same as I used in my study[7] of complement fixation and of carbohydrate reactions.

[1] Wien. klin. Wchnschr., No. 32, 1897.
[2] Ann. de l'Inst. Pasteur, 1899, 13, p. 179.
[3] Ibid., p. 406.
[4] Compt. rend. Soc. de biol., 1906, 9, p. 846.
[5] Jour. Exp. Med., 1907, 16, p. 329.
[6] Jour. Med. Research, 1907, 16, p. 329.
[7] Jour. Infect Dis., 1910, 7, p. 159.

It is essential that the antigen, that is, the culture filtrate, should not contain the slightest amount of serum of any kind. I have used Thallmann's broth in which gonococci were allowed to grow for 4 weeks at 37 C. and then removed by centrifugation at 2000 revolutions a minute. Carbolic acid was added to the supernatant fluid in the proportion of 0.5%.

The serum of various patients has been used. Antigonococcus serum was obtained from rabbits injected with gonococci grown for from 24 to 48 hours on horse blood agar. Carbolic acid, 0.5%, was added as a preservative.

The serum of healthy persons, of normal rabbits, of patients with other diseases than gonorrhea, and other immune serums were used also, as well as antigens prepared with the meningococcus, the micrococcus catarrhalis, the staphylococcus, and the colon bacillus.

In making the tests the serum is diluted and thoroughly mixed with the antigen, the mixture being left at room temperature for 24 hours.

Experiments with the serum of 6 healthy young men who never had had gonorrhea gave absolutely negative results with all the antigens except, in one case, a slight precipitate formed in the mixtures with the staphylococcus and colon bacillus antigen.

TABLE 1

CROSS PRECIPITIN REACTIONS WITH THE SERUM OF RABBITS INJECTED WITH GONOCOCCI

Immune Rabbit Serum	Gonococcal Strains *							
	A	B	C	D	E	F	G	H
A	200	200	100	50	200	50	100	200
B	200	200	200	100	200	50	200	200
C	200	200	200	50	200	50	200	200
D	50	50	100	200	500	200	50	50
E	200	200	200	50	200	200	200	200
F	100	100	100	200	200	200	50	50
G	100	100	100	50	100	50	100	100
H	200	200	100	50	200	50	200	200

* The figures give the highest dilution of the serum in which distinct precipitation took place.

In 2 cases of acute gonorrheal urethritis in men the result was negative. In the case of a man, 28 years old, who had chronic gonorrheal urethritis since he was 18, a precipitate was obtained with the antigens of all the 8 strains of gonococci in a dilution of the serum of 1 : 10, and with 5 of the antigens in a dilution of the serum of 1 : 50.

In another case of chronic and recurrent urethritis in a man 38 years old, 6 strains reacted with the serum in a dilution of 1 : 100. There was a slight precipitate with the meningococcus antigen at a serum dilution of 1 : 10.

In 2 cases of chronic gonorrheal urethritis the reaction was wholly negative. The serum of a woman with chronic gonorrheal endometritis gave precipitate with all the gonococcal antigens to the same degree, dilution of 1 : 25, but in 2 other women there was no precipitate.

In the case of a man, aged 35, the subject of chronic urethritis and gonococcal arthritis, the serum reacted with all 8 antigens but most markedly with the same 6 as previously noted.

In the case of 2 patients who had had gonorrhea some years previously followed by complete cure, the results of precipitin tests were negative.

In the case of 3 patients with other diseases than gonorrhea and without any history of having had gonorrhea the precipitin tests were also negative.

The results of these observations show that specific precipitins may develop in the course of chronic gonococcal infections, but not regularly; in these cases the reaction may not be equally well marked with different antigens, but the difference is only a quantitative one. Occasionally human serum contains a small amount of precipitin for meningococci.

The serum of healthy rabbits does not contain any gonococcal precipitin.

The serum of rabbits immunized with the 8 different strains of gonococci used in these experiments gave reactions as shown in Table 1. These are the serums that I used in my complement fixation tests.[7] As shown by this table the 8 strains of gonococci appear to fall in 2 groups, but the difference between the 2 groups is merely a quantitative one, as no strain was found to be wholly refractory. Strains D and F were precipitated by all the serums in dilutions of at least 1 : 50, and Serum D and F caused precipitates with all the strains of gonococci.

In no case did any of the antigonococcus serums form precipitates in control tubes with salt solution or with extracts of the meningococcus, M. catarrhalis, the staphylococcus or the colon bacillus. The serum of rabbits injected with the meningococcus, M. catarrhalis or with the colon bacillus also gave only strictly specipe reactions; serum of horses injected with typhoid or dysentery bacilli, or the serum of an ox injected with dysentery bacilli gave no precipitates with gonococcal extracts. Normal horse, ox, goat, and dog serums were also without action. In the mixtures of gonococcal extract and antigonococcus serum the precipitates form slowly, requiring about 5 hours at room temperature before any cloudiness could be detected even in the stronger serum mixtures.

SUMMARY

Normal serums, human, rabbit, horse, ox, goat, dog, as a rule, do not contain precipitins for gonococcal protein.

The serum of patients with gonococcal infection may contain specific gonococcal precipitates, but this seems to be the case when the infection has been severe and has lasted for some time. The serum of patients with typhoid fever or other infections, not gonococcal, does not contain gonococcal precipitin.

The serum of rabbits injected with 8 strains of gonococci contained precipitin each one for all the different gonococci, but in the case of 2 strains the extract gave precipitates in lower dilutions with the serum of the other 6 rabbits than with homologous serum and their serum gave precipitates with the other 6 extracts in lower dilutions than the homologous extract. The serum of rabbits injected with the meningococcus, M. catarrhalis and the colon bacillus gave no precipitates with gonococcal extracts.

Apparently antigonococcus serum is not as rich in specific precipitin as in agglutinins and complement-fixing bodies, but the precipitin reaction distinguishes more definitely between gonococci and meningococci, as well as M. catarrhalis, than complement fixation.

BRILLIANT GREEN AS A BACTERICIDAL AGENT FOR THE PURIFICATION OF VACCINE VIRUS

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AND

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As far as we know Churchman[1] is the only investigator who has made observations on the effect of strongly bactericidal anilin dyes on vaccine virus. He gives no details stating simply that the potency of vaccine virus was unimpaired by staining with gentian violet.

We were led to investigate the influence of anilin dyes on the potency and bacterial content of vaccine virus because of the frequent presence of gas-producing anaerobic bacilli giving positive reactions with the official hygienic laboratory tests for the presence of B. welchii; and because of the continued viability of these bacilli in spite of the glycerole and carbolic acid added to purify and preserve the virus.

Brilliant green was used because of its wider range of bactericidal action[2] as compared with the violet dyes and especially because of its action on bacilli of the colon group.

The data here presented in this article show that brilliant green has little, if any, influence on the potency of vaccine virus, and added to the present glycerol-carbolic preservative, gives a more efficient and more rapidly bactericidal preservative.

The accompanying tables give the bactericidal results obtained with virus artificially inoculated with the bacterial types considered most objectionable in virus.

Brilliant green, therefore, added to heavily inoculated glycerol-carbolic virus led to a rapid reduction of the inoculated bacteria and sterilization in 6 days, whereas with glycerol-carbolic alone the reduction was slower and sterilization was not complete in 6 days.

In this connection it is well to note the persistence of this type when encountered in glycerol-carbolic virus as prepared for distribution.

[1] Proc. Soc. Exper. Biol. and Med., 1914, 11, 55.
[2] Krumwiede, C., and Pratt, J. S.: Jour. Exper. Med., 1914, 19, 501.

TABLE 1

Vaccine Virus to Which Was Added One-Fourth of its Volume of a Heavy Milky Suspension of Staphylococci and Streptococci. Kept in Refrigerator Between Tests

Dates Tested 0.1 C.c. Plated, Blood-agar	Inoculated Glycerol-Carbolic Virus Containing Brilliant Green; Concentration, 1:10,000	Inoculated Glycerol-Carbolic Virus; Saline to Same Volume as Preceding
July 22, 1915	Plates, solid very closely crowded fine colonies	Plates as preceding
July 23	33% reduction in number of colonies	No evident reduction
July 24	40% reduction	5% reduction
July 26	99% reduction	15% reduction
July 27	Three colonies total on 3 plates	50% reduction
July 28	No growth	98% reduction

TABLE 2

Virus Containing Large Numbers of Bacilli Giving Positive B. Welchii Tests Was Further Inoculated with Cultures of a Similar Bacillus Isolated from Previous Virus. The Virus Was the Routine Glycerole-Carbolic Suspension

Glucose-Fermentation Tubes Inoculated	Inoculated Virus	Inoculated Virus, Brilliant Green 1:5000	Inoculated Virus, Brilliant Green 1:10,000
At time of mixing	90% gas	No gas; probably dye inhibition	100% gas
After 2 days	95% gas	No gas	No gas
After 6 days	60% gas	No gas	No gas

TABLE 3

Virus Heavily Inoculated with Tetanus Spores Obtained from Broth Culture Sediment

Tested	Inoculated Virus	Inoculated Virus, Brilliant Green 1:5000	Inoculated Virus, Brilliant Green 1:10,000
At once	Tetanus bacilli isolated	Tetanus bacilli isolated	Tetanus bacilli isolated
After 248 days	Tetanus bacilli isolated	Tetanus bacilli isolated	Tetanus bacilli isolated

Virus 2260, collected April 14, 1915; B. welchii types still present, March 1, 1916.

Virus 2261, collected April 28, 1915; B. welchii types found May 6, 1916, not found June 9, 1916.

Virus 2262, collected May 12, 1915; B. welchii types found May 16, not found June 9, 1916.

Virus 2263, collected May 12, 1915; B. welchii types found June 11, 1916.

No quantitative estimations were made, but based on the amount of growth obtained in the primary "shake" cultures made from the virus, numerous spores were present at the end of the experiment.

Although tetanus spores are evidently highly resistant, this is indirectly an advantage over the present preservative. If tetanus bacilli were present, they should be easily recovered due to the killing of the associated bacteria and spores, especially of B. welchii types which otherwise by overgrowth nullify the accepted tests for the presence of tetanus bacilli or spores.

Before instituting a series of tests of viruses with and without brilliant green, it seemed well to determine whether the carbolic acid was of added value when the dye was employed. With the carbolic acid, the disappearance of some undetermined types of cocci was more rapid. Comparative tests also revealed that a concentration of at least 1 : 10,000 of the dye was necessary to obtain bacteriologic sterility in a relatively short time. A greater concentration, 1 : 5000, was not appreciably more effective, and as at this concentration there was some indication of injury to the vaccine virus itself, practically all the subsequent observations were made with a concentration of 1 : 10,000.

Evidently brilliant green when added to glycerol-carbolic virus markedly hastened its purification. As frequently the first test on the brilliant green virus gave negative cultures the actual earliest day on which a bacteriologically sterile preparation was obtained cannot be stated. Because of this, the following comparison of averages is distinctly in favor not of the virus thus treated, but in favor of the glycerol-carbolic virus. Thus, with the brilliant green virus the average day on which gas was not noted was 11 days; growth not noted 24 days; with the glycerol-carbolic virus, gas was still positive after an average of 33 days, and growth was still positive after an average of 119 days.

The accompnying chart gives a summary of the potency tests of vaccine virus with and without brilliant green. The concentration of brilliant green employed was 1 : 10,000.

Duration of potency of vaccine virus with and without brilliant green.

Double line=100% positive takes of highest quality. Single line=100% positive takes of moderate quality. Break in both lines=less than 100% takes. Break in one line=break in quality of takes. "Takes"=primary vaccinations of children. (We are indebted to Drs. Samuel Parnass and Julius Blum who carried out the clinical tests on children). +=brilliant green added. O=no brilliant green, glycerol-carbolic virus.

TABLE 4

Comparative Rate of Purification of Glycerol-Carbolic Virus with and Without Brilliant Green. Tested by Inoculation of Glucose Broth in Fermentation Tubes. Day Noted of Last Positive and First Negative Test for Gas Production; of Last Day of Clouding and First Day of Absence of Growth. Tested About Every 7 to 14 Days. Gas Noted As Its Development Is an Indication of the Presence of B. Welchii Types

Virus Number	Brilliant Green 1:10,000				Brilliant Green not Added			
	Gas	Days	Growth	Days	Gas	Days	Growth	Days
14	+	2	+	11	+	30	+	92
	—	3	—	20	—	39	——	——
15	+	10	+	82*	+	93	+	266
	—	21	—	91	——	——	——	——
16	——	——	——	——	+	43	+	45
	—	9**	—	9**	——	——	——	——
17	——	——	——	——	+	4	+	45
	—	9**	—	9**	—	17	——	——
19	——	——	——	——	+	44	+	44
	—	16**	—	16**	—	44	——	——
21	+	1	+	24	+	32	+	199
	—	8	—	29	—	42	——	——
22	+	3	+	26	+	14	+	193
	—	10	—	32	—	21	——	——
23	——	——	——	——	+	13	+	156
	—	11**	—	11**	—	31	—	171
24	——	——	——	——	+	4	+	147
	—	12**	—	12**	—	13	——	——
25	——	——	——	——	+	71	+	71
	—	9	—	9	—	94	—	94
26	——	——	——	——	+	28	+	112
	—	13	—	13	—	55	——	——
27	——	——	+	28	+	14	+	57
	—	13	—	37	—	21	—	71

* Only organism after 2 months, small coccus.   ** No previous tests.

Although variations in the degree of "takes" are sometimes in favor of the virus, without brilliant green the reverse also occurs. On the whole, there is no evidence of the dye exerting any appreciable deleterious influence, especially if the duration of potency is considered. Probably some of the variations in results are dependent on inherent variables in the method of testing.

As a control on the foregoing human vaccinations, vaccinations were carried out on rabbits with dilutions of treated and untreated vaccine, as explained in Table 5.

The results recorded in Table 5 show no influence of the dye on the total content of virus after exposure for 40 days.

We have employed brilliant green to preserve the seed-virus collected from calves vaccinated with human virus as well as to preserve

TABLE 5

POTENCY TESTS ON RABBITS VACCINATED WITH VACCINE VIRUS WITH AND WITHOUT BRILLIANT
GREEN DYE 1:10,000.   VIRUSES 5 AND 6 WERE EXPOSED TO BRILLIANT
GREEN, MARCH 31, 1916

Virus Number and Date of Test	Without Brilliant Green Dilution of Virus	Results	With Brilliant Green 1:10,000 Dilution of Virus	Results
June 9, 1916				
Virus 5	Undiluted	Good confluent take	Undiluted	Good vonfluent take
Virus 5	Diluted 1:10	Good confluent take	Diluted 1:10	Good confluent take
Virus 5	Diluted 1:100	Good confluent take	Diluted 1:100	Good confluent take
Virus 5	Diluted 1:1000	Good confluent take	Diluted 1:1000	Good confluent take
June 9, 1916				
Virus 6	Undiluted	Good confluent take	Undiluted	Good confluent take
Virus 6	Diluted 1:10	Good confluent take	Diluted 1:10	Good confluent take
Virus 6	Diluted 1:100	Good confluent take	Diluted 1:100	Good confluent take
Virus 6	Diluted 1:1000	Good confluent take	Diluted 1:1000	Good confluent take

the seed-virus from rabbits vaccinated with this human-calf virus. This rabbit virus is used to vaccinate calves in order to obtain virus for general distribution; the interpolation of the rabbit avoiding calf to calf inoculation which commonly has a deleterious effect on its potency.

In Table 6 the seed exposed and the time of exposure are given.

The addition of brilliant green to the seed virus had no effect on the potency of the final virus. In no instance was any appreciable diminution noted in the intensity of the "take" nor in the amount of yield of virus from the animals vaccinated.

At first the vaccine virus was treated with brilliant green by adding 1 part of a 1:1000 solution of the dye to 9 parts of the finished

TABLE 6

TYPE OF SEED EXPOSED, TIME OF EXPOSURE AND RESULTS

Human-Calf Virus; Number and Date of Exposure	Rabbit Seed; Number and Date of Exposure	Calf Vaccine; Number and Date Vaccinated	Duration of Potency of Calf Vaccine in Days
	1 July 18, 1916	18 Aug. 9, 1916	Still good at 300 days
8 June 10, 1916	2 July 25, 1916	19 Aug. 24, 1916	Still good at 294 days
		22 Sept. 29, 1916	Still good at 301 days
	1 Oct. 9, 1916	24 Nov. 17, 1916	Still good at 253 days
		25 Dec. 7, 1916	Still good at 233 days
12 June 10, 1916	3 Nov. 8, 1916	27 Dec. 21, 1916	Still good at 218 days
		28 Jan. 23, 1917	Still good at 175 days
	4 Jan. 16, 1917	32 Feb. 8, 1917	Still good at 168 days
	5 Feb. 5, 1917	33 Feb. 21, 1917	Still good at 155 days

Explanation: Numbers refer to animal collections. From left to right gives passages of each collection from calf to rabbit to calf.

glycerol-carbolic virus. Later the pulp from the calf was ground up in a glycerol-carbolic solution containing 10% less water and this volume of brilliant green solution (1 : 1000) added, thus avoiding any dilution of the finished product as compared with the routine glycerol-carbolic virus.

In observing the results of vaccinations made with the brilliant green-treated virus, there has been some indication that the secondary inflammation was reduced. Possibly this effect could be enhanced by subsequent applications of the dye-solution to vaccination, limiting in this way the multiplication of the skin cocci.

The sterilization of the virus by brilliant green may find an important practical application in that intermediate sterilization would allow calf to calf vaccination, without loss in potency of the virus. Without bacterial sterilization such a procedure quickly leads both to degeneration of the virus and decreasing yields, due apparently to a great extent to the overgrowth of the calf bacterial flora enriched by the transfer of this flora from calf to calf. At present this overgrowth is avoided by obtaining the seed for calf vaccination from rabbits.

The use of brilliant green alone, although somewhat less efficacious than in combination with carbolic acid, may be of service in obtaining a bacteriologically sterile virus for attempts at cultivation. The observation of Churchman and Russell[3] that certain animal tissues grow readily in concentrations of gentian violet which are bactericidal is suggestive in this connection.

CONCLUSIONS

Brilliant green in a concentration of 1 : 10,000 has no appreciable effect on the potency of vaccine virus. This amount of dye when used in combination with the glycerol-carbolic solution usually employed markedly hastens the rate of reduction of the bacterial content, rendering most preparations bacterially sterile in from 2-4 weeks.

Although the dye cannot be relied on to kill tetanus spores should they be present, no difficulty should be encountered in demonstrating their presence after the associated bacteria are destroyed.

The results obtained warrant its practical application to vaccine virus for general distribution or at least in emergencies when the virus must be employed shortly after collection from the calf.

The use of brilliant green gives a simple method hitherto not available for obtaining a bacteriologically sterile but fully potent virus for experimental purposes.

[3] Proc. Soc. Exper. Biol. and Med., 1914, 11, 123.

A DETERMINATION OF THE NUMBERS OF HISTIDIN DECARBOXYLATING ORGANISMS IN THE FECES IN DEMENTIA PRAECOX AS COMPARED WITH THE NUMBERS IN NORMAL FECES

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Several investigations, among which is the work of Berthelot and Bertrand,[1] have shown that an organism possessing remarkable powers for decarboxylating various amino and other organic acids can be isolated by special methods from the intestinal tracts of certain individuals. This organism Berthelot and Bertrand called B. aminophilus intestinalis, and described as similar culturally to B. mucosus, but differing from the latter in this marked property of decarboxylating certain COOH containing acids.

In the case of one of the amino-acids, namely, histidin, the remaining portion of the molecule after decarboxylation is the corresponding amin, histamin. And B. aminophilus, when grown in a histidin-containing medium, produced this intensely toxic histamin in a concentration such that these investigators were able to kill guinea-pigs with doses as small as 0.001 c.c.

Dale and Laidlaw[2] have shown that plain muscle responds with violent contraction when brought in contact with extreme dilutions of histamin. In fact, a method, based on this remarkable physiologic action of histamin, has been developed and is now in common use in testing for the presence of this substance.

With the evidence from these investigations as a starting point, a theory regarding the etiology of dementia praecox has been advanced by Bayard Holmes in recent articles.[3] He believes that the organism discovered by Berthelot and Bertrand, or at any rate, some organism capable of transforming histidin into histamin, is present in the intestinal tracts of dementia praecox patients. Such organisms, by utilizing

the histidin set free in the intestinal tract by digestion of protein, could then produce the intensely toxic amin, histamin. The histamin would be absorbed by the blood, and then, because of this powerful physiologic action on plain muscle tissue, would cause a constriction of the ring of Cannon. This constriction would bring about a condition of cecal stasis, which by holding the food residues in the cecum for several days, would favor the development of more histamin-forming organisms, with more formation and absorption of the toxic base, and further construction of the ring of Cannon—the various phenomena forming a vicious cycle, with constant poisoning of the system with histamin. This prolonged poisoning of the various organs of the body might show itself in various ways, but its action on the nervous system is assumed to be more or less specific and to give rise to the symptoms seen in dementia praecox.

In a recent article,[4] the author of this theory describes how the condition of cecal stasis, lasting from 4-5 days in dementia praecox, has been demonstrated as a fact, by fluoroscopic methods. Furthermore, he asserts that histamin has been isolated by chemical methods, and that bacteria capable of producing histamin from histadin are present in the intestinal tracts of such patients.

The investigation reported in this article was undertaken to determine the significance of this latter statement, namely, to compare the relative numbers of histamin forming organisms in a given weight of stool from dementia praecox patients with the relative numbers of similar organisms from a similar weight of stool from normal subjects.

TECHNIC

At the outset it was considered necessary to isolate the suspected variety or varieties of organisms which, when grown in pure culture in a histidin containing medium, can produce a detectable quantity of histamin, and to establish some of the biochemical characteristics by which such organisms could be distinguished from other associated varieties. The method of enrichment, used by Berthelot and Bertrand, was used, and after an examination of the stools of some dozen or more patients and controls, an organism was finally isolated which readily converted histidin into histamin. It was found to be similar to the organism described by Berthelot and Bertrand. A loopful of feces was inoculated into the histidin containing medium and after enrichment in two successive transfers in this medium, the organism, when streaked on Endo plates, appeared as a rapidly growing, white, semi-translucent, mucuslike colony. It is a capsulated, nonspore bearing, gram-negative, nonmotile, facultative anaerobic bacillus, producing on agar slants a mucuslike growth similar to B. mucosus.

[4] Med. Council, 1917, 22, p. 31.

It was found later, however, that the latter may be distinguished from this histamin forming variety when grown in glycerol broth. The histamin forming variety is unable to ferment glycerol.

Incidentally, this organism was isolated, not from one of the patients, but from a supposedly normal control, and in spite of the fact that the enrichment method was used in the search for it, it did not appear in any of the other stools examined.

It was then thought possible that by devising some selective plating medium on which colonies of this organism could be recognized directly, it would be a simple matter to determine the relative numbers of the organism in each sample of stool when plated directly on such medium. Accordingly a medium, prepared after the following method, was used for this purpose: 0.1 gm. of histidin-hydrochlorid, 0.025 gm. KNO₃, 0.002 gm. CaCl₂, and 0.02 gm. K₂SO₄ are dissolved in a small flask in about 50 c.c. of distilled water; 0.02 gm. MgSO₄ dissolved in 10 c.c. of water is added to this; 0.5 gm. K₂HPO₄ dissolved in water is then added, and the entire amount made up to 100 c.c.; 0.8 gm. agar is then washed and added to the solution, and the mixture sterilized at 15 lbs. for 15 minutes. The agar is dissolved by this process, and when mixing the medium, care should be taken not to form air-bubbles in the mixture. Now add 1.2 c.c. of a 10% solution of Na₂SO₃ and titrate the solution by running in N 10 HCl, 1 c.c. at a time, until the medium is neutral to litmus paper. Three drops of a saturated alcoholic solution of basic fuchsin are now added and the medium after being thoroughly mixed is poured into plates. The precipitate of Ca₃(PO₄)₂ will not interfere with the purpose for which the medium is to be used and should not be filtered off, since even the slight solubility of this solid is sufficient, as well as necessary, for the growth of this organism.

On such a medium the organisms of the intestinal tract were soon found to divide themselves readily into 5 different groups: (1) those which are unable to develop in the presence of the basic fuchsin, and which are promptly eliminated from the group to be studied; (2) those which, on a medium containing such elementary substances, are unable to produce visible colonies; (3) those which are able to produce only very minute colonies; (4) those which grow readily, but which, even in the absence of sugars, are able to restore the color of the fuchsin and thus appear as red colonies, and (5) equally rapidly growing colonies which do not become red, but which show varying degrees of translucence.

In the case of the organism in question, which shall be referred to as B. aminophilus, a peculiar characteristic was revealed which was not duplicated by any other variety encountered. It appeared after 24 hours at 37 C. as a colony of from 2-3 mm. in diameter, having the appearance of a clear colorless drop of water. This remarkable translucence, which even B. mucosus could not imitate, was retained indefinitely, so long as the agar was kept from drying up.

To determine the efficiency of this medium in disclosing the presence of B. aminophilus in a given sample of stool the following experiment was made:

Two grams of an average sample of stool from a patient suffering from catatonia and other pronounced symptoms of dementia praecox, were thoroughly mixed with a mortar and pestle. One gram was then removed to a watch glass, while the remaining portion in the mortar was inoculated with 2 loopfuls of B. aminophilus, and mixed again. This portion was then removed to a 2nd watch glass. One half of each portion was again removed to separate mortars and diluted and mixed with 5 c.c. of water. Dilutions 1 : 100,000 were now made of each sample, and plated on 10 plates of the histidin agar described, by placing 1 drop of the dilution in the center of the plate and spreading it over the agar surface with a sterile bent glass rod. After 48 hours at 37 C. each of the 10 plates inoculated with the sample to which had been added the 2 loopfuls of the B. aminophilus culture, showed a count of approximately 150 colonies per plate. About 10% of these were of the B. aminophilus type, which appeared as clear colorless watery colonies, and which were subsequently identified as B. aminophilus. The other 10 plates, inoculated with the sample which had not been mixed with the B. aminophilus culture, showed about the same number of colonies, but none of those with the watery appearance of the B. aminophilus type could be found on any of the 10 plates. Furthermore, plain agar plates, inoculated at the same time with the same dilution of this sample, revealed the fact that this selective medium permitted only about one twentieth of the intestinal flora to develop visible colonies. In other words, out of 10 plates, representing 150 bacteria each, or 1,500 in all, not one colony of B. aminophilus could be found, and if these 1,500 colonies represent only one-twentieth of the living bacteria of the intestinal flora, it means that if B. aminophilus was present in this sample of stool, it was present in a ratio of less than 1 : 30,000 of other living bacteria in the sample. Obviously, for practical purposes, the organism may be considered absent. The stools from 6 other patients were then examined by the same method, and the same negative results obtained in every case.

In order to determine if the absence of the organism may have been due to the fact that, although present in the upper portion of the intestinal tract, it might have perished later in the lower portion of the colon, the following tests were made: The 2 samples of 0.5 gm. each remaining on the watch glasses, were held anaerobically at 37 C. for 48 hours, and the process of plating then repeated. It was found that, although under conditions approximating those existing in the colon, and which presumably might be unfavorable for the organism, B. aminophilus is not destroyed, but on the other hand, increases out of proportion to the rate of increase of the other histidin utilizing varieties.

Throughout the investigation, however, a continual search was kept up for other possible varieties which could produce histamin from histidin, and for this purpose the medium was modified by leaving out the Na_2SO_3 and fuchsin, since either of these two substances might suppress the growth of some histamin forming varieties. Furthermore, plates incubated over periods of two weeks, some under aerobic, and

others under anaerobic conditions, revealed no other histamin forming variety.

The special enrichment method used by Berthelot and Bertrand, and Mellanby and Twort,[5] are obviously unsuited for determining the relative number of a given organism in mixed culture, since a single living individual of the given organism, among millions of other bacteria, might eventually gain the ascendency in the enrichment medium and then be easily isolated. The reason for this is probably the following: The medium used by Berthelot and Bertrand, for example, when inoculated with such a heterogeneous mixture of varieties as is found in feces, rapidly becomes alkaline. This alkalinity becomes the limiting factor in the multiplication of the different varieties, and quite naturally, those varieties possessing the greatest tolerance for this alkalinity will be permitted to further increase their numbers long after the other varieties have been overcome by the alkaline conditions of the culture. Given then, an organism possessing a high rate of multiplication plus a high degree of tolerance for hydroxyl-ions, such an organism, if present, will naturally gain the ascendency in a few transfers in such a medium. In this case, B. aminophilus is such an organism. It has a rate of growth perhaps not exceeded by any other organism commonly found in feces, and in addition is able to continue its metabolism in an alkalinity represented by a P_H^+ of 8.7. With these two characteristics in its favor, it should be able to compete with the other varieties in the mixed culture, and to gain the ascendency after a few transfers in this medium. Then by plating the growth obtained in the last transfer, B. aminophilus should be easily isolated. Such a method, used, as previously stated, by Berthelot and Bertrand, was attempted a second time. One set of transfers was held under aerobic, and another under anaerobic conditions, and finally streaked on histidin agar plates. In most cases the method resulted in yielding a pure culture of some type, from each stool examined, usually of the lactis aerogenes variety. Occasionally, B. mucosus variety was found and in one case a rapidly growing, vigorous liquefier of gelatin was obtained. B. cloacae was isolated once. In no case, however, out of 38 different samples of stools taken from dementia praecox patients and normal controls, was the B. aminophilus or any other histamin forming variety discovered. The method used for detecting histamin formation in these cultures was the method devised by Dale and Laidlaw,[2] and

[5] Jour. Physiol., 1912, 45, p. 53.

claimed by these and other investigators to be delicate to 1 part of histamin in 250,000,000 parts of water. Some other method, however, may possibly lead to more successful results in demonstrating the presence of histamin forming varieties in the stools in dementia praecox.

But even granting that such organisms are present in these patients it would still remain to be shown what significance their presence may have, by showing that the same organism is not present in the intestinal tracts of normal subjects, which so far as the literature reveals, has not been done. The failure to find appreciable numbers of histamin forming varieties in the stools in dementia praecox, however, does not prove that histamin should not be present in such stools. It is commonly known that two different varieties of organisms, neither of which in pure culture can produce a given chemical change, when grown in mixed culture may produce that change. On the other hand, their presence, even in large numbers, would not justify the assumption that histamin should also be found there, as was shown most clearly by the following experiment:

One hundred c.c. of the histidin containing medium used by Berthelot and Bertrand was inoculated with B. aminophilus, and incubated at 37 C. for 3 days. The culture was then killed by boiling, neutralized with N/10 HCl and divided into 3 parts. Part 1 was inoculated with feces from a praecox patient; Part 2 with B. aminophilus, and Part 3 was left uninoculated. All 3 parts were held at 37 C. for 3 days, and then tested for the presence of histamin. Part 1 gave a negative, Part 3 a positive, and Part 2 a still stronger positive reaction for histamin.

These results show 3 things: First, that histamin formed by B. aminophilus can be destroyed by other intestinal bacteria; second, that B. aminophilus probably can use the histidine carboxyl (COOH) group only, and third, that the alkalinity of the culture becomes the limiting factor in the production of histamin, by the bacterial method, and that with successive neutralizations of the culture, a larger yield of this valuable product should be obtained. (Regarding the latter point, an investigation of the value of such a method is now being made.)

From the work of Kendall[6] and others, it was predicted that when B. aminophilus is supplied with other available sources of carbon, for example, dextrose, it would not use the carboxyl (COOH) portion of histidin, and therefore would not form the amin. Dextrose was added

⁶ Jour. Med. Research, 1911, 25, p. 117.

to the histidin containing medium and after incubating with B. aminophilus for three days, the culture was examined for the presence of histamin. A negative reaction was obtained, thus verifying the prediction. The same result was obtained when tyrosin was used in place of histidin. This same experiment was done by Mellanby and Twort,[7] but these investigators interpreted the results to mean that histamin formation was prevented because the acid reaction, resulting from the fermentation of the carbohydrate, was unfavorable for the decarboxylating action of the bacteria. They came to this conclusion because a tube of medium, rendered acid purposely, at the time of inoculation, also failed to yield histamin. Unfortunately, they do not state how much acid was used, although they admit that growth in a sugar free medium resulted in a final alkaline reaction. As a matter of fact, B. aminophilus when grown in a medium purposely made acid to a reaction of as high a P_H^+ as 5.2, will promptly turn the medium alkaline, and histamin formation proceeds normally. Higher concentrations of hydrogen-ion do prevent histamin formation, but only because B. aminophilus then fails to grow. In general, it may be said that B. aminophilus does not form the amin through choice, but only by dint of the circumstances imposed on it, namely, by being forced to subsist on a medium containing only the one source of utilizable carbon, histidin.

It is noteworthy that histamin formation goes on in a medium showing a marked alkalinity, in a range of P_H^+ inimical to the activities of most intestinal bacteria. Under this condition, the histamin formed in the intestine could accumulate, since the other varieties, which at higher ranges of P_H^+ would promptly demolish the histamin molecule, are now inactive. It is also noteworthy that stools from persons suffering from constipation invariably show a marked alkalinity, a P_H^+ as low as 8.6 in some cases observed. In such a stool, provided other sources of utilizable carbon are absent, formation of the amins (not necessarily histamin only) could proceed, and these, if not absorbed, could accumulate. But that histamin can be isolated by chemical means from the stools of dementia praecox patients, as stated by the author of this theory, has no significance in establishing the validity of the theory until it is also proved that the same substance is not present in the highly alkaline stools of other people. As a matter of fact, histamin has been shown to be a normal constituent of the intestinal

[7] Jour. Physiol., 1912, 45, p. 57.

mucosa. The works of Bayliss and Starling,[8] and Barger and Dale[9] are significant in this connection. Their investigations, undertaken separately and for different purposes, proved that histamin is easily extracted from fresh intestinal mucosa. They make no attempt to explain its presence here, but simply point to the fact that histamin is a constant constituent of the lining of the intestine. Furthermore, during this investigation, an examination of a water extract of a stool from one of the control subjects gave a strong Pauly reaction, and an oxytocic reaction for tyramin (which is formed from tyrosin by the same organism forming histamin from histidin).

With regard to the cecal stasis said to occur in dementia praecox by the author of this theory, it may be said that in 4 cases of dementia praecox examined by me, and 3 other cases of which roentgenograms had been made, no indications of a cecal stasis such as would result from a constriction of the ring of Cannon was seen, and certainly nothing like a cecal stasis of from 4-5 days' duration. The most that could be said of these cases was that in two of them, the roentgenograms revealed a retention of the barium meal, not in the cecum, but in the pelvic colon, for a period somewhat longer than normal; and even this condition, which was nothing more than the ordinary constipation commonly seen in dementia praecox, was only temporary.

SUMMARY

An organism similar to the one described by Berthelot and Bertrand has been isolated; it is shown to differ from B. mucosus by its inability to form gas and acid in glycerol broth.

This organism, called B. aminophilus, does not form histamin from histidin when more available source of carbon of the carbohydrates is present.

A selective plating medium for isolating this organism from mixed culture has been devised. By this medium it was shown from an examination of samples from 38 different stools, that B. aminophilus ordinarily is not found in the human intestinal tract in sufficient numbers to be isolated by direct plating of a quantity of feces representing from 20,000 to 100,000 living bacteria.

[8] Jour. Physiol., 1902, 28, p. 335.
[9] Ibid., 1911, 45, p. 499.

THE ROLE OF SPECIFIC FATS IN COMPLEMENT FIXATION

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In previous articles[1] it has been shown that the fats peculiar to the gonococcus served as antigen in complement fixation tests for gonorrhea to a degree better than aqueous or salt solution suspensions or autolysates of the germs. To test whether the antigen emlpoyed in the serum test for syphilis also depends for its value on a specific fat content a long series of tests was made[2] from which it was determined that such was the case, the specificity depending on a definite fatty composition or configuration of the lecithin-like substance.

Since then the work has been extended to include antigens for cholera, typhoid and tuberculosis.

For the tests with the fat of the cholera vibrios there were used an Austrian strain of virulent organisms and guinea-pig immune serum. Mass cultures on neutral nutrient agar in Roux flasks were washed off in small amounts of distilled water, completely saponified by potassium alcoholate with heat under 100 C. and the soaps recovered as fatty acids, after acidification, by solution and washing in dry ether. The ethereal solutions were then concentrated to dryness in vacuo, the fatty acids taken up in alcohol, converted into the sodium salts by sodium carbonate, the salts repeatedly recrystallized from alcohol, dried, weighed and redissolved in absolute alcohol. These salts were free from protein.

The salts were preferred to the fatty acids themselves as previous experience had shown them to yield better complement fixation. A series of fixation tests was then carried out on the immune cholera serum of guinea-pigs, the antigen consisting of the alcoholic solution of the specific cholera fats of which solution 1 c.c. contained 0.002 gm., the hemolytic system being the sheep-rabbit system, and the complement

fresh guinea-pig serum. All the reagents were titrated in the usual manner. The tests showed that complement was bound in the first incubation by the specific fat-sensitizer (amboceptor) complex formed, for the subsequent addition of sensitized sheep cells showed no hemolysis, or the addition of sensitized cholera vibrios gave no Pfeiffer phenomenon. The following specimen protocols illustrate these facts, but before considering the application of this principle to the organisms mentioned it is advisable at this point to regard its relation to cells other than bacteria. If it be true that definite fat complexes of bacterial cells are able to replace the cells themselves as antigen in the serum test we should expect an analogous phenomenon to obtain with respect to the red blood cells serving as antigen in the hemolytic system. That such is the case was shown by suitable experiments. When the fats specific to the red blood cells (sheep) or their stromata,[3] in the form of an alcoholic solution of the sodium salts (or with alcohol-ether extracts) were incubated with sensitizer and complement, the complement was found to be absorbed, so that on the subsequent addition of sensitized corpuscles, or of sensitized cholera vibrios, there was no hemolysis or Pfeiffer phenomenon.

Corpuscle Antigen

Alcoholic solution of fat antigen, prepared as outlined, 1 c.c. contained 0.002 gm. Antigen neither hemolytic or anticomplementary in working doses. Dose employed, by weight, 0.0000112 gm. (sufficient to bind total complement).

Fresh guinea-pig serum, 5 standard drops of one-tenth dilution in salt solution, varying with titration, used as complement.

Antisheep-rabbit serum, 1 standard drop, 0.02 c.c. or 2 units.

Corpuscle suspension—2% suspension in salt solution of fresh washed sheep corpuscles; dose 0.5 c.c.

Sensitized sheep corpuscles—0.5 c.c. washed cells added to 1 c.c. inactivated immune serum and kept at 37 C. for 1 hour, then washed with salt solution; 2% suspension in salt solution.

Total volume in each tube brought 1 c.c. with salt solution. First and second incubations 1 hour each at 37 C.

Control tubes contained: (1) Antigen + corpuscles; (2) sensitizer + corpuscles; (3) complement + corpuscles; (4) corpuscles + salt solution; (5) complement + salt solution 37 C. 1 hour, then sensitized corpuscles 0.5; (6) sensitizer + complement + salt solution 37 C. 1 hour, then corpuscles suspension 0.5 c.c.

Exper. 1.—Seven determinant tubes contained each, antigen, sensitizer, complement, salt solution. Incubation for 1 hour at 37 C.; 0.5 c.c. corpuscles suspension added, again incubation for 1 hour.

Result: No hemolysis in any tube. Complete hemolysis in control Tubes 5 and 6.

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[3] Bordet: Studies in Immunity, Collected and translated by Gay, 1909.

Exper. 2.—Same as 1, substituting sensitized corpuscles for unsensitized. No hemolysis in any tube; complete hemolysis in Controls 5 and 6.

Exper. 3.—Substituting sensitized cholera vibrios, suspended in salt solution, for sensitized corpuscles. No Pfeiffer phenomenon in any determinant tube. Pfeiffer phenomenon complete in Controls 5 and 6.

Cholera Antigen

Heavy suspension in salt solution of cholera vibrios; suspension of sensitized vibrios; doses by titration 2 drops. Cholera antiserum (guinea-pig), inactivated, dose 1 drop, 2 units or 0.02 c.c. Cholera antigen, alcoholic solution, dose 1 drop, by weight same as in Expers. 1, 2 and 3. Other details same as in foregoing experiment, substituting cholera suspension for corpuscle suspension.

Exper. 4.—Six determinant tubes containing antigen, sensitizer, and complement salt solution. Incubation 1 hour at 37 C.; 2 drops vibrio suspension added; 2nd incubation for 1 hour. No agglutination or Pfeiffer phenomenon in determinant tubes. Agglutination and Pfeiffer phenomenon in Controls 5 and 6.

Exper. 5.—Same as 4, substituting sensitized cholera vibrios for unsensitized in 3 determinant tubes, and substituting sensitized sheep cells for vibrios in the last 3 tubes. No Pfeiffer phenomenon or hemolysis in any determinant tube; complete Pfeiffer phenomenon in Tube 5; complete hemolysis in Tube 6.

TYPHOID ANTIGENS

Similar experiments were carried out with the specific fats of the typhoid bacillus. The antigen was prepared as outlined previously (1000 sq. in. agar surface culture yielded 0.310 gm. of the sodium salts), using 5 strains of the organisms, and the serum came from animals immunized against typhoid, paratyphoid A and B, B. coli, and from persons sick with or convalescent from typhoid fever. These experiments showed that the typhoid fats were also specific, and that they gave fixation with paratyphoid B and with colon antiserums according to the subjoined details.

Exper. 6.—Antigen, alcoholic solution, 1 c.c. contained 0.002 gm.

Sensitizers, immune rabbit serum for typhoid bacilli, paratyphoid A and B, B. coli and human antityphoid serum, all inactivated.

Complement, sensitized vibrio and corpuscle suspensions, and other details the same as in the preceding experiments.

Rabbit: Antityphoid serum + +
          Antiparatyphoid A serum −
          Paratyphoid B serum + +
          Anticolon serum, +

Human serum: Typhoid convalescent + +; agglutination +
               Typhoid convalescent +; agglutination +
               Typhoid convalescent +; agglutination +
               Typhoid fever, 3rd week + +; agglutination +
               Typhoid fever, 3rd week + +; agglutination +
               Typhoid fever, 2nd week + +; agglutination +
               Typhoid fever, 2nd week + +; agglutination +

Human serum: (continued)
               Normal —
               Normal —
               Syphilitic —; Wassermann ++
               Syphilitic —; Wassermann ++
               Gonorrheic —; complement fixation ++
               Gonorrheic —; complement fixation ++
               Scarlatinal —
               Scarlatinal —

Cross experiments wherein the specificity of the fat complexes was shown, and serving as negative controls in the preceding experiments, consisted in substituting the various antigens for the specific one in mixtures of the different immune serums and complement. These showed that complement is not bound save with the appropriate specific antigen and sensitizer, but that with antigens derived from germs of one species fixations occur with serums for related species. Indeed the range of the fats of the cells thus far studied has been shown not to be so great but that occasional fixation is given with an unrelated serum, by reason of the overlapping.

ANTIGENS OF BACILLUS TUBERCULOSIS

With regard to tuberculosis, experiments with the specific fatty antigen have shown an exception. At first sight it might appear that the failure of this antigen to bind complement in the presence of serum of patients suffering with tuberculosis constituted an exception fatal to the idea of the specificity of fats, especially in view of the results of serum tests in the hands of Craig and others[4] who have used antigens consisting of alcohol treated bacilli, aqueous suspensions and autolysates. On further consideration, however, it has become apparent that the exception tends rather to prove the rule, since I have been unable to show the presence of antibody in any serum from tuberculous patients. Moreover it has not been possible to obtain results at all comparable to those of the authors mentioned, using as antigens the various suspensions and preparations of tubercle bacilli made as nearly as possible according to their methods. As is shown in the accompanying table of representative tests we obtained results equally as good with suspensions of other organisms, and are forced to conclude that tests for antibody in the serums of tuberculous patients by the method of complement fixation with any antigen are unavailable

⁴ Craig: Jour. Am. Med. Assn., 1917, 68, p. 723. Corper: Jour. Infect. Dis. 1916, 19, p. 315. Corper and Sweeney: Jour. Am. Med. Assn., 1917, 68, p. 1598. Miller and Zinnser: Proc. Soc. Exper. Biol. and Med., 1916, 13, p. 134.

and unreliable because of its frequent absence. This contention is supported by many facts in the literature.[3]

The following antigens have been employed and found to be of no value.

Alcoholic extracts of moist tubercle bacill at 25 C.
Alcoholic extracts of moist tubercle bacilli at 78 C.
Concentrated broth and 50% alcohol.
Alcohol and ether extracts of dry tubercle bacilli.
Tubercle poison (Vaughan), alcoholic solution.
Tubercle residue (Vaughan), aqueous suspension.
Tubercle extract, ether (Vaughan), alcohol soluble portion.
Tubercle extract, ether (Vaughan), alcohol-ether soluble portion.
Tubercle wax, melting point 45 C., alcohol-ether solution.
Tubercle alcohol, from saponified wax; hot alcohol solution.
Tubercle alcohol acetic ester alcohol solution.
Total fatty acids, alcohol solution.
Total sodium salts of fatty acids, alcohol solution.
Aqueous suspensions of tubercle bacilli.
Aqueous autolysates (salt solution).

Controls

Syphilitic antigen.
B. Leprae (Duval), aqueous suspensions.
B. Typhosus, aqueous suspensions.
B. Anthracis, aqueous suspensions.
Bacillus X, from scarlatina, aqueous suspension.

The aqueous antigens were titrated so as to obtain a dose lying in the very narrow zone between the anticomplementary point and that giving no fixations at all. The alcoholic and ethereal extracts appeared to be anticomplementary in all doses. The addition of cholesterol increased this action, while the addition of the tubercle alcohol diminished it. By itself the alcohol possessed neither hemolytic, anticomplementary, or antigenic value in ordinary doses. The specific tubercle fats were not of themselves anticomplementary but were made so by the faintest traces of cholesterol. This was contrary to experience with the other antigens of Expers. 1-7, in which the addition of cholesterol gave sharper and more clearly cut inhibitions than without.

Examples of the results are given in Tables 1-4, in which ++ denote complete inhibition of hemolysis, + partial inhibition, and ± doubtful inhibition.

These results have been selected from many hundred tests as representing the best that is to be said for these antigens. The reactions were never sharp and clear cut. In a majority of instances it

TABLE 1

Tests with Tubercle Antigens *

Serums		Autolysate of Tubercle Bacilli	Sodium Salts of Fatty Acids from Tubercle Bacilli	Fatty Acids of Tubercle Bacilli
Active tuberculosis	1	±	—	—
	2	—	—	±
Chronic tuberculosis	1	+	+	++
	2	++	++	+
Syphilis		±	—	++
Normal	1	++	—	++
	2	—	—	—

* In this and the accompanying tables, ++ denotes complete inhibition of hemolysis, + partial inhibition, ± doubtful inhibition, and — absence of inhibition.

TABLE 2

Tests with Tubercle Antigen

Serums		Aqueous Suspensions of Tubercle Bacilli	Sodium Salts of Fatty Acids from Tubercle Bacilli	Fatty Acids of Tubercle Bacilli
Active tuberculosis	1	+	++	—
	2	±	++	—
	3	+	±	—
	4	++	±	+
	5	—	++	±
	6	±	—	±
Chronic tuberculosis	1	+	+	++
	2	+	±	±
	3	+	+	++
	4	+	++	++
	5	—	++	+
	6	++	++	+
Syphilis	1	—	—	—
	2	±	+	++
	3	—	±	—
Normal	1	++	±	++
	2	—	—	—
	3	—	++	++
	4	—	+	—
	5	++	+	+
	6	+	—	—

TABLE 3

Tests with Tubercle Antigens and Suspension of Bacillus X

Serums		Aqueous Suspension of Bacillus X from Scarlatina	Aqueous Suspension of Tubercle Bacilli	Autolysate of Tubercle Bacilli	Aqueous Suspension of Tubercle Bacilli	Autolysate of Tubercle Bacilli
Active tuberculosis	1	+	−	±	+	−
	2	−	±	+	+	−
Chronic tuberculosis	1	+	−	+	+	−
	2	+	±	±	+	+
Syphilis		−	+	++	++	+
Normal	1	+	±	+	+	+
	2	+	−	−	±	+

TABLE 4

Tests with Tubercle and Other Antigens

Serums		Aqueous Suspension of						Wassermann	Gonococcus	Typhoid Bacilli (Agglutination)
		Tubercle Bacilli	Bacillus Leprae	Tubercle Residue	Typhoid Bacilli	Bacillus X Scarlatina	Anthrax Bacilli			
Active tuberculosis	1	−	−	−	++	++	−			
	2	−	±		−	++	−			
	3	+	+	±	++	+	−			
	4	+	−	±	±	−	−			
	5	±	+	±	±	+	±			
	6	±	±	−	±	+	−			
Chronic tuberculosis	1	±	−	−	+	++	−			
	2	−	−	−	±	++	±			
	3	±	++	−	+	+	−			
	4	+	+	−	−	−	−			
	5	±	++	−	++	++	+			
	6	+	±	+	+	++	−			
Typhoid fever	1	±	−	+	++	+	−		·	+
	2	±	±	−	−	±				+
Controls	1	+	±	+	−	−	++	++	++	
	2	±	±	++	−	−	++	++	++	
	3	+	±	+	−	+	±	−	++	
	4	−	−	−	−	−	±	−	−	
	5	±	±	++	−	−	++	−	+	
	6	+	+	+	−	±	++	−	−	

was necessary to set a time limit to the second incubation in order to obtain readings, and when the incubation was allowed to proceed in a manner consistent with the requirements of the controls by far the greater number of the determinants showed complete hemolysis. It is distinctly bad practice to accept such readings since they represent delayed hemolysis and not fixation of complement. When a positive reaction occurs with the syphilitic, the gonorrheic, the typhoid or other antigens mentioned there is no question about it. The cells settle to .the bottom of the tubes and no amount of prolonged incubation or shaking alters the readings.

DISCUSSION

The antigenic rôle of lipoids in the immune reactions of agglutination, precipitation and complement fixation has been noted by many writers [3,5] especially those dealing with the serum reaction for syphilis. The presence of nitrogenous and other substances in the so-called lipoids has, however, justly supported the criticism that the impossibility of excluding such substances left the antigenic value of the fats themselves much in doubt. To the pure specific fats this criticism is not applicable, especially since in one instance at least an artificial fatty antigen from pure materials has been substituted,[6] and unpublished work indicates that many of the fatty complexes of bacterial and other cells can be assembled artificially.

It is not claimed and it is not likely that such fatty compounds represent the true state of the cell fats as they exist in the cells nor is it asserted that the form in which they are used in the reaction of fixation is the best possible form. Be this as it may it is claimed nevertheless that the complexes are available for antigenic use, that they represent the specific fat aggregates of the cells, and that the state in which they are used, in addition to their chemical configuration, is essential to their successful operation. The sensitizer-fatty antigen aggregate which favors the adsorption of complement is more readily brought about when the fats are in a certain physical state. For instance, the same fats as neutral fats will yield no complement adsorption while as fatty acids or as the alkali salts of the fatty acids the adsorption readily occurs. That the special or specific configuration of the fats is the key to the process of fixation is shown by the

[5] Bang and Forsmann: Beitr. z. chem. Phys. u. Path., 1906, 8, p. 238. Warden: Jour. Am. Med. Assn., 1917, 68, p. 432.

[6] Warden: Jour. Am. Med. Assn., 1917, 68, p. 932.

fact that the slightest deviation from this configuration impairs the specificity of the test.

The rôle of these complexes of pure cell fats which are regarded as specific for each cell will be made clearer by holding a few well established facts in mind. We must regard the immune reaction of the fixation complement, the formation of specific precipitates, and the cytolytic properties of immune serums as embracing essentially the same phenomenon, that of the lytic action of complement on the surface of the cell sensitized by the appropriate sensitizer following its adsorption on the sensitized cell (Bordet). In other words, the action is unquestionably one directed on cell surfaces, or aggregate surfaces, or stromata in the sense of Bordet. Many bacterial and other cells, particularly those retaining the Gram stain, are known to contain fats at their surfaces, and from what is known of the chemico-physics of cells in general it appears to be safe to assume that the fats in one form or another constitute a large bulk of the stroma and surfaces, although by no means or necessarily to the exclusion of other substances such as protein and cholesterol, or other sterols.

Since now the presence of the cells themselves has been shown to be unnecessary and their functions assumed by the fats specific for those cells in the immune reaction we must conclude that sensitized antibody forms with the fat a specific aggregate which adsorbs complement in a manner wholly analogous to the process as worked out by Gay[3] for cell and other specific precipitates.

The application of these principles to the study of typhoid serum reactions will continue and form the subject for further communication. At the same time the idea is being extended to include other organisms, particularly the various strains of the pneumococcus.

SUMMARY

The value of antigens in serum tests for the presence of antibody in gonorrhea, syphilis, typhoid fever, and cholera appears to depend on fatty complexes of definite chemical arrangement or configuration which represent the fatty content of the several micro-organisms causing those diseases.

The reactions of the test are surface reactions and depend on the physical state of the antigen.

The serum test as applied to tuberculosis is fallacious and unreliable probably because of the absence of sensitizers in the serums.

EXPERIMENTAL VACCINE SHOCK AND THE RESISTANCE TO TUBERCULOSIS

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Recently Baldwin and L'Esperance[1] described observations concerning the effect of intravenous vaccine injections (protein shock) on tuberculous guinea-pigs, and particularly the unusual alteration in the tissue reaction to the tuberculous process from that commonly observed in these animals. One of us,[2] dealing with the relation of the serum ferments and antiferment to tuberculosis, has suggested the possibility that the observations of Baldwin and L'Esperance might be due to the increase in the antiferment titer of the treated animals, which would tend to protect the tubercle from digestion by the strongly active proteolytic serum ferments, and thereby prevent the rapid dissemination of the infecting bacilli from the foci.

English observers early noted and suggested the clinical use of the increase in the antiferment titer following ordinary vaccine injections, and Bruce[3] described the increase in the weight curves of patients who were under vaccine treatment for a considerable period of time, a fact that Mircoli[4] has demonstrated experimentally in normal animals following tuberculin injections.

Inasmuch as any intoxication ordinarily results in an almost immediate increase in the excretion of nitrogen, it is evident that the increased weight the various authors have noted following vaccine injections must involve the bringing into play of some compensatory mechanism, through the action of which the organism is able to retain a greater amount of nitrogen than before the vaccine shock. There is considerable evidence that the increase in the antiferment is one of the factors involved in this mechanism,[5] particularly in the smaller animals in which the serum is rich in proteolytic ferments.

[1] Jour. Immun., 1917, 11 p. 283.
[2] Petersen, W. F.: Arch. Int. Med., 1917, 20, p. 716.
[3] Brit. Med. Jour., 1910, 1, p. 430.
[4] Pathologica, 1914, 5, p. 118.
[5] Jobling, J. W., and Petersen, W. F.: Ztschr. f. Immunitätsforsch., 1915, 24, p. 219.

In the following experiment we have followed the weight curves, the nitrogen excretion, as well as the serum alterations of animals (rabbits, 4 in the series) which were twice given intravenous doses of a colon vaccine. The animals, of approximately the same age and weight, were given the 1st injection of 20 million killed colon bacilli on the 8th day at 10 a. m., serum being taken from the ear vein before the injection, 6 hours after and on the following day. A temperature reaction of approximately 3.5 C. followed the injection. A second injection was made on the 30th day of the experiment; this time only 10 million organisms were used, serum being taken before and 2 days after the injection. Following this injection the temperature reaction was slightly over 1° C.

In Chart 1 the curves illustrate the averages for all the animals, the solid line representing the vaccine animals, while the dotted line represents the curve of the normal animals.

It will be observed that the weight curve of the vaccine animals decreased slightly after the 1st injection, remaining below that of the controls for the entire period to the time of the 2nd injection. After this the vaccine animals continued to gain slightly over the controls until at the close of the experiment the vaccine animals weighed approximately 140 gm. more than the controls.

The nitrogen excretion, as determined by the Kjeldahl method, indicated a total excretion of 18.4 gm. for the vaccine animals as compared with 15.2 gm. for the controls. The increase in nitrogen excretion apparent in the curve during the latter part of the experiment was due to a greatly increased food ration given the animals during the latter half of the experiment.

When these findings are correlated with the changes in the proteolytic balance of the serum we find that the vaccine animals responded to the vaccine shock with a considerable augmentation of the antiferment, which, even after 3 weeks, was still at a higher level than at the beginning of the experiment, and was increased after the 2nd shock, from 35 to 47%. The curves for the normal animals also showed some increase during the course of the experiment, but this spontaneous change was much less than that following the vaccine injection.

In determining the proteolytic titer of the serum we have used a method suggested by Flatow,[6] that is, the incubation of casein solution with the serum for a definite time. The digestion that results is the

[6] München. med. Wchnschr., 1914, 61, p. 1500.

index of the relative proteolytic balance existing between the proteolytic ferments present (protease and ereptase) and the antiferment. It is not to be regarded as the index of the total amount of protease which
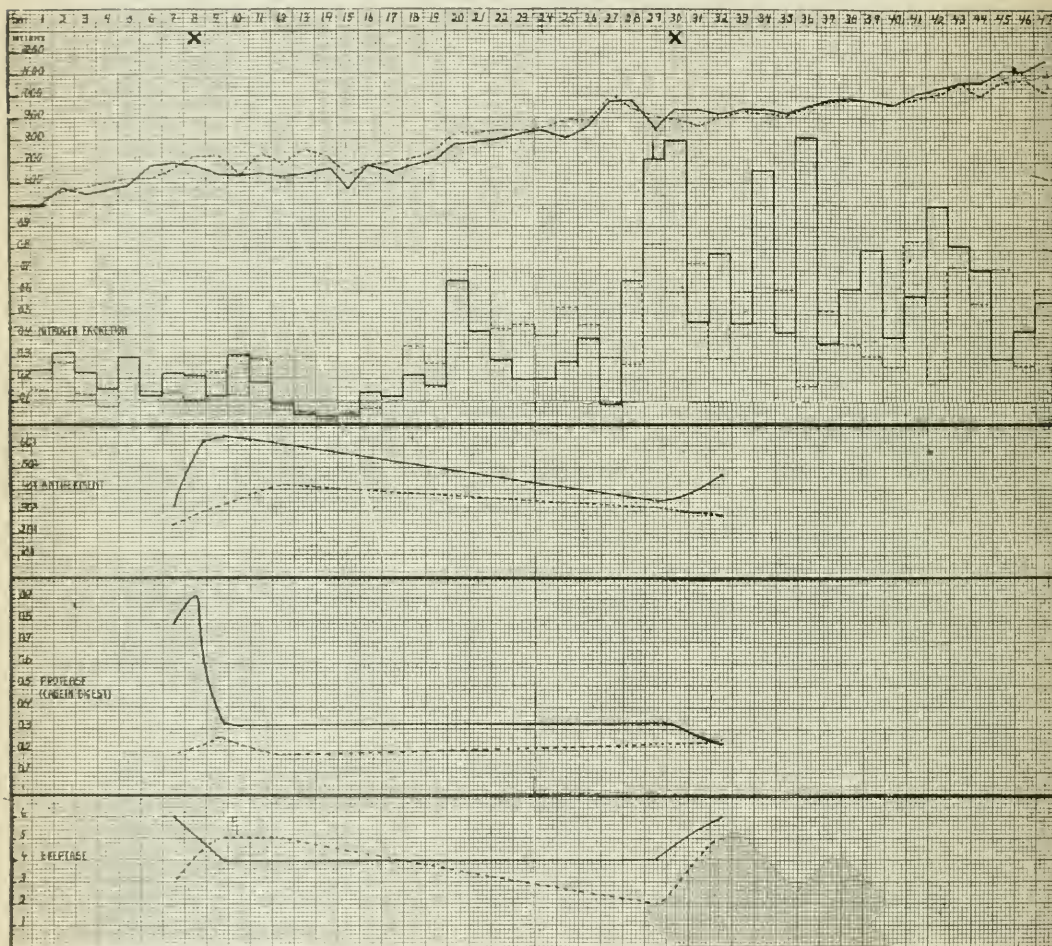
Chart 1.—Effect of vaccine shock (injections indicated by X) on the weight, nitrogen excretion, antiferment, proteolytic index and ereptase titer of rabbits. The curves illustrate the averages for all the animals. The solid line records the results secured in the experiments with the vaccine animals and the dotted line shows the curve obtained with the control animals.

is brought into effect only when the antiferment is removed by some means, either through the acidification of the serum or the removal of the antiferment by the lipoid solvents. In making this determination

we have used 0.5 c.c. of serum and incubated for 48 hours with 2 c.c. of a 1% solution of neutral casein. The coagulable proteins were then removed and the noncoagulable nitrogen determined by the Folin method.

The differences between the boiled control and the tube which had been incubated at 47 C. have been charted in terms of 10th mg. It will be observed in the chart that after a short rise immediately following the vaccine injection, the proteolytic index of the serum was markedly diminished from the originally rather high titer, and the decrease was continued after the 2nd vaccine injection as well. The control animals showed very little alteration during the course of the experiment.

The short rise apparent immediately after the vaccine injection is due to the marked mobilization of protease which takes place following shock of this kind.[7] When this has disappeared, the effect of the increase in the antiferment becomes evident and depresses the proteolytic titer to a point far below the original titer prevalent before the vaccine shock.

The ereptase titer illustrated in the last curve of the series showed no characteristic alteration, although it too was decreased in the vaccine animals, while the control animals showed a slight increase in the titer.

DISCUSSION

An analysis of the serum alterations that take place following vaccine shock in these animals would indicate that one of the fundamental changes effected had to do with the decrease in the proteolytic index that results from the increase in the antiferment. With this observation as a basis we can understand that, despite the increased nitrogen elimination incident to shock, the animal tends to retain nitrogen to a greater degree than before the vaccine shock, so that such animals actually show a net gain in weight after the first effects of the shock have worn off.

It is from the standpoint of the resistance of the organism to tuberculosis, however, that we believe these alterations to be of greater interest. The progress of tuberculous infection in the guinea-pig and the rabbit is one of relatively great rapidity, the protecting tissue reaction which, in man, very probably forms the great bulwark against the progress of the disease, is largely lacking; indeed, fibrosis about the foci that might be comparable to the condition in man is rarely

[7] Jobling, J. W., and Petersen, W. F.: Jour. Exper. Med., 1915, 21, p. 239.

met with. We must keep in mind that these animals differ from man in the relatively great concentration of proteolytic ferments in the serum which are balanced by a narrow margin of antiferment, a condition the exact reverse of that found in man. In the smaller animals any tendency toward fibrosis is apt to be encountered by the digestive effect of the serum, and it is only when the antiferment is increased that a greater development of fibrous tissue is noted, as has been described by Baldwin and L'Esperance. This relation of the antiferment (and of the lipases) to the resistance of tuberculosis has recently been emphasized by Fernandez[8] in an interesting paper.

In view of the considerable differences in the proteolytic activity of the serum of the smaller experimental animals and man, it might seem warranted to observe considerable caution in the interpretation of experiments relating to tuberculous resistance in these animals unless proper recognition of the difference is kept in mind.

[8] Siglo méd., 1917, 64, p. 376; abstr. in Jour. Am. Med. Assn., 1917, 69, p. 159.

ON THE RELATION OF THE SERUM EREPTASE (PEP-TIDASE) TITER TO THE CLINICAL COURSE IN PNEUMONIA

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During the course of recent years a number of investigators have emphasized the possibility that a definite relation exists between the inception of the crisis in pneumonia and the activation of the proteolytic ferments in the area involved. Edsall and Pemberton[1] in particular have advanced this idea and endeavored to make the logical clinical application of hastening autolysis as a therapeutic measure in cases of delayed resolution. Later, Jobling[2] studied the serum ferments and antiferment during the course of pneumonia, noting the fact that just preceding the crisis protease was demonstrable in the serum, while the antiferment began to diminish from the high titer prevalent throughout the early part of the disease.

The fundamental idea underlying the several studies in this direction has been that, apart from the intoxication arising directly from and incident to the growth of the pneumococcus, toxic split products were probably absorbed from the exudate, which could be considered a mass of foreign protein undergoing slow digestion before the crisis. Active autolysis once underway, only the lower and nontoxic products of digestion would be absorbed, and the environment for the continued proliferation of the pneumococcus would become unfavorable, as Almagia[3] has suggested. In this phenomenon the primary reaction is of course local and cellular, involving the liberation of sufficient leuko-protease from leukocytes to overcome the inhibiting factors. These latter are very probably the antiferment of the serum and the exudate.

Of the varieties of proteolytic ferments involved in such a digestive process, the titer of the peptidase or ereptase during the course of pneumonia has not been studied. This form of enzyme activity would

seem particularly favorable in the pneumonic process, inasmuch as, theoretically at least, it could lead only to a detoxication through the complete destruction of toxic protein fragments, and the activity of the ferment is not subject to any inhibitory agents in the serum, as is the true protease.

Normal human serum contains peptidase and maintains a relatively uniform titer, augmented possibly after feeding by an increased supply coming in through the lymph stream.[4] For purposes of study practically all recent workers have employed glycyltryptophan, first introduced by Neuberg and Fischer,[5] but inasmuch as this was not to be had we made use of a 10% solution of Witte peptone, neutralized to litmus, and determined the splitting of the peptone by the usual bromid test. The titer of the serum was determined by a simple dilution method, all the serum for any given patient being titrated at the same time. During the course of the work the serums of 18 cases of pneumonia were titrated for the ereptase and antiferment content, but only 4 are charted here, inasmuch as they are quite representative for the series.

Case 1.—A white man, 43 years old, entered the hospital March 14, 1917, after an illness of 36 hours. Diagnosis: Lobar pneumonia of the lower right lobe; course uneventful; recovery by lysis by the 10th day. Chart 1.

The ereptase (peptidase) titer, it will be noted, remained uniformly low for the first 3 days examined, increased to approximately 3 times the former titer on the 8th day of the illness and then again diminished. The antiferment, on the other hand, showed the usual early rise, then declined during the period of lysis, but again increased.

Case 2.—A colored man, 27 years old, entered the hospital April 18, 1917, having been ill for 2 days previous. Diagnosis: Lobar pneumonia of the lower left lobe; aortic regurgitation. On the 3rd day evidence of consolidation of the right lower lobe was noted and the patient became progressively worse, dying on the 9th day of the disease.

It will be observed (Chart 2) that the titer curve of the ereptase was in this case the reverse of the preceding, showing a decrease after the 3rd day which persisted to the end. The antiferment, meanwhile, increased progressively.

Case 3.—This case (Chart 3) illustrates a febrile course altered by intravenous vaccine shock which brought the temperature to normal for several hours but did not alter the course of the disease in any other way. The patient was admitted, April 5, 1917, having been ill for the 5 days preceding. Diagnosis: Lobar pneumonia of the upper left lobe. April 7, he was given a small (25 million) intravenous dose of typhoid vaccine, following which he had a slight chill and a fall in temperature to normal the following morning. The afebrile period was transient, however, the former level being regained the same day. This was followed by a normal lysis.

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4 Davis, B. F., and Petersen, W. F.: Jour. Exper. Med., in print.

5 Deutsch. Arch. f. klin. Med., 1909, 97, p. 499.
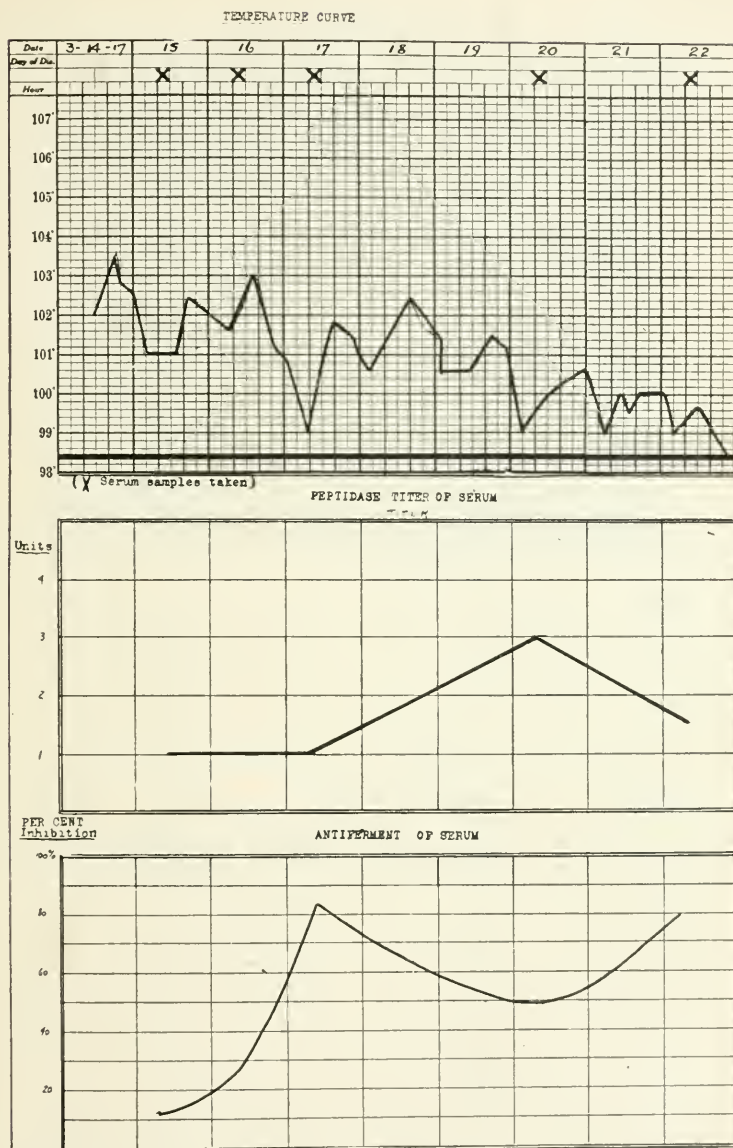
Chart 1.—The ereptase (peptidase) and antiferment titer during the period of lysis in pneumonia.
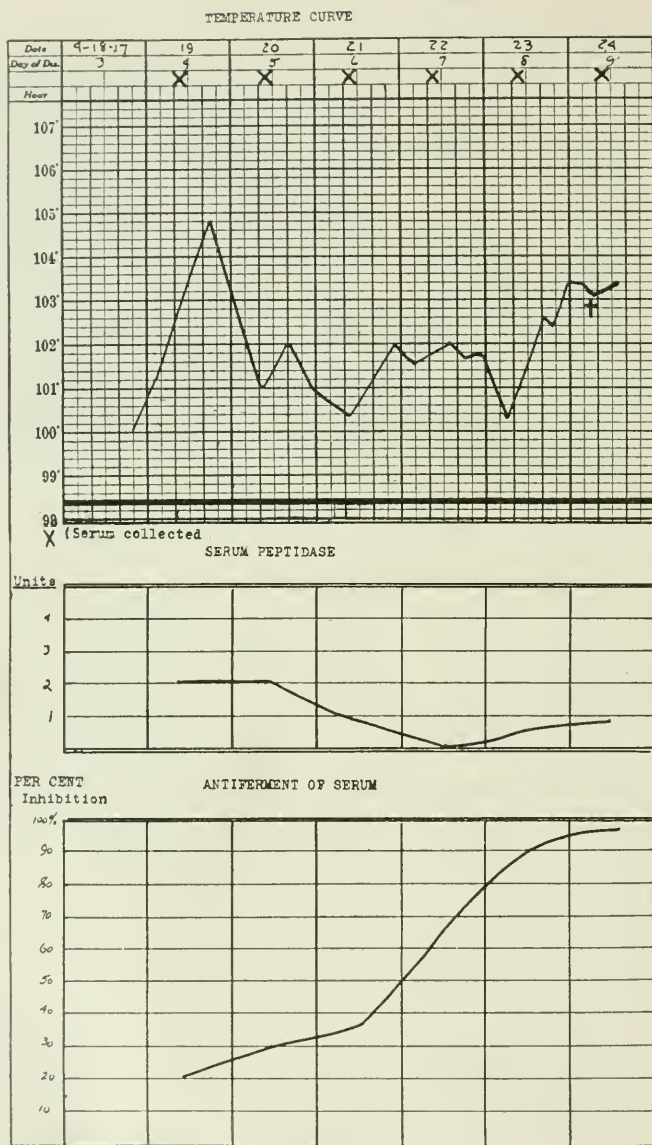
Chart 2.—The ereptase titer and antiferment in pneumonia terminating in death.
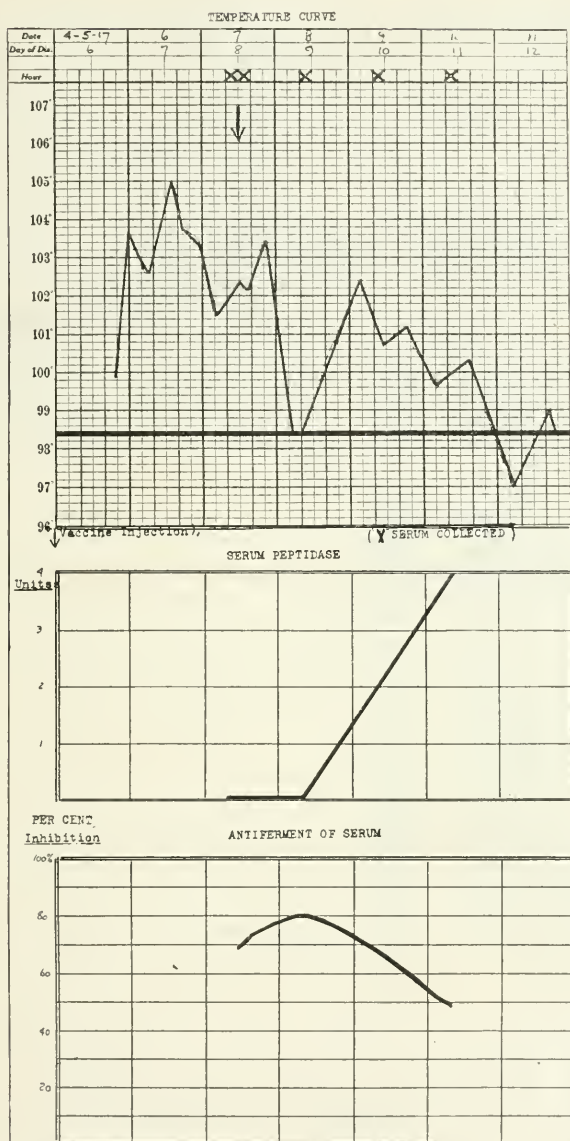
Chart 3.—Serum ereptase and antiferment titer in pneumonia subject to vaccine shock.
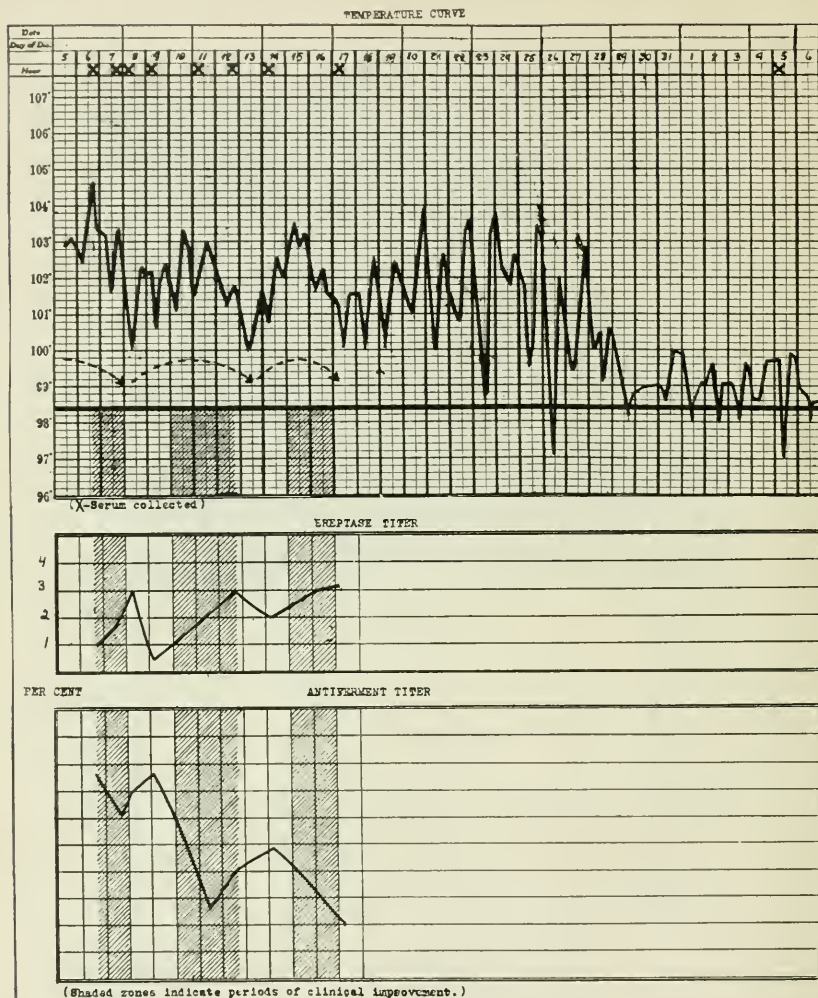
Chart 4.—The relation of the clinical condition during pneumonia to the serum ereptase and antiferment.

The serum titration was interesting in that no ereptase was present before or after the vaccine injection but the titer increased spontaneously during the period of lysis, the antiferment diminishing during the same time.

CASE 4.—Possibly the relation between an increased ereptase titer of the serum and clinical improvement is best illustrated in the following case: A white man, 34 years old, entered the hospital, March 5, 1917, with a diagnosis of lobar pneumonia of the lower right lobe of 3 days duration. The course of the disease was quite protracted. By the 4th day after admission the temperature had fallen and the patient was clinically improved when a consolidation of the

middle lobe occurred. The temperature rose for several days, then declined to the 8th day. At about this time the lower left lobe became involved, which entailed another rise in temperature, followed by a period of partial improvement; a long period of great temperature fluctuation then set in. A month after admission the patient was still running an irregular febrile course although greatly improved. At this time a final blood examination was made. In Chart 4 the periods of temperature elevation as each new lobe was involved are quite well defined and are indicated by the dotted curved lines below the temperature chart. The shaded zones have been added to indicate the periods of intercurrent improvement.

It will readily be seen that a definite increase in the ereptase titer takes place during the period of improvement, only to decrease with the more unfavorable clinical condition. The antiferment on the other hand, invariably tended to diminish during the favorable periods and to increase with the increase in the lung involvement.

DISCUSSION

The 4 charts used to illustrate the relation of the ereptase titer of the serum are quite characteristic for the changes observed in the balance of the cases studied. In pneumonia that terminates by crisis or by lysis an increase in the ereptase titer invariably precedes or accompanies the clinical change, whereas in the cases that terminate fatally such an increase has not been observed, the titer usually remaining below that of the normal individual. While the activity of this enzyme is not interfered with by the serum antiferment, nevertheless the latter almost invariably diminishes when the ereptase increases, making the conditions most favorable for proteolysis. It is possible that with the use of glycyltryptophan (making a shorter incubation period possible for the test) the determination of the titer of the ereptase may be of clinical use for purposes of prognostic orientation.

In view of the possible, indeed probable, rôle of the ereptase in acting as an agent in detoxicating the partially hydrolyzed protein fragments by bringing about their complete lysis, it is attractive to conceive of this ferment as definitely influencing the pathologic process under consideration. It will not be possible, however, to draw any such conclusion until we are able to increase the ferment activity of the serum by some method and thereby study the effect of wilfully produced alterations of the titer on the disease. Until that is possible it will not be warranted to regard the change other than an accompaniment of the altered clinical state, with the possibility in mind that it may be one of factors involved in bringing about the alteration.

A TRYPANOSOME OF PANAMANIAN CATTLE AND A METHOD FOR CONCENTRATING TRYPANOSOMES IN PERIPHERAL BLOOD

Oscar Teague and Herbert C. Clark

From the Board of Health Laboratory, Ancon, Canal Zone

We have been able to show that a large percentage of the beef cattle brought to Panama City from various parts of the Republic of Panama carry in their blood a large trypanosome. Since these animals are apparently in good health, the presumption is that the trypanosome is nonpathogenic; however, another possibility is that the trypanosome is pathogenic, but that the cattle have developed an immunity against it.

The trypanosome could not be found in direct smears of the blood, but developed in cultures grown at 26 C. For this purpose 2 c.c. of the freshly drawn and defibrinated blood were added to 10 c.c. of nutrient broth containing 0.85% of sodium chlorid. The trypanosome can usually be detected in the upper layer of the sediment (the leukocytic layer) of the culture after 24-48 hours' incubation. On the 3rd or 4th day they are generally present in large numbers, occurring in clusters with the flagella extending outward and the posterior ends embedded in a central clump of leukocytes. The trypanosomes remain alive and actively motile in these cultures for 30 days and longer. After a few days, however, marked changes in morphology occur; along with forms resembling trypanosomes, but varying markedly in size, are others like crithidia, some oval flagellated bodies, and even spermatozoan-like forms.

Obviously the cultures were unsatisfactory for studying the morphology of the trypanosome. Since smears from the blood of cattle known to be positive by the culture method were negative and laboratory animals inoculated with the cultures of the trypanosome and with cattle blood did not show trypanosomes in their blood, there appeared to be only one possible way of accurately determining the morphology of the trypanosome, namely, by destroying the red cells and then concentrating the trypanosomes in laked blood by centrifugation. Several different methods were tried to accomplish this purpose, the following one being finally chosen.

The freshly drawn blood is whipped with wooden sticks in an enamel-ware cup and the defibrinated blood is poured through wire gauze to remove bits of fibrin. Ten c.c. of the blood are added to 10 c.c. of distilled water, and the two are thoroughly mixed. This was done at the slaughter house. Hemolysis of the red cells takes place in a few minutes. As soon as we reached the laboratory, the blood was centrifugated, the supernatant fluid was poured off and the sedi-

ment was emulsified in the 2 or 3 drops of fluid remaining. Smears from this sediment showed well preserved trypanosomes in almost every instance, from 1-10 or 12 or more to a slide having been found. A drop of the sediment examined under a cover-slip occasionally showed a trypanosome, which remained actively motile for more than an hour after the distilled water had been added to the blood. There is a distinct advantage in defibrinating the blood before laking the red cells, as a smaller amount of distilled water is required and the sediment after centrifugation seems to be smaller in amount.

After we had developed this concentration method, a calf that had been inoculated 7 days previously with a large dose of cattle blood showed trypanosomes in its peripheral blood in such numbers that they were very readily found in direct smears. This furnished us with additional material for the study of the morphology of the trypanosome.

In size and morphology the trypanosome agrees with the description of Tr. theileri. In the films from the calf's blood the largest trypanosome measured by us was 4.5μ wide by 54μ long exclusive of the flagellum which was 13.5μ long; the smallest was 20.4μ long and 3.6μ wide with a flagellum measuring 16.2μ. They were usually about 45μ long by 3.5μ wide with a flagellum about 14μ. The posterior end is long drawn out and pointed. The undulating membrane is well developed. The blepharoplast is round and is generally situated one fourth the length of the body of the trypanosome from the posterior end. Some of the very small trypanosomes have the blepharoplast disproportionately near the posterior end and the flagellum is relatively longer than in the larger individuals. The blepharoplast has most often a marginal location. The nucleus stains well and lies nearly equidistant from the two ends of the trypanosome.

The trypanosomes from adult cattle obtained by the concentration method previously described were larger than those in the blood of the experimental calf and showed marked differences in their staining characteristics. That these differences were not the result of the treatment with distilled water was shown by the fact the trypanosomes in the calf's blood, when so treated, stained in the same manner as those in direct smears from the blood. The cattle trypanosomes measured from 42μ to 80μ long without the flagellum and from 3.6μ to 5.4μ wide. The nucleus is in most instances represented by a rectangular pale space occupying the entire width of the trypanosome and situated approximately equidistant from the 2 ends. In rare cases a faintly staining oval was found and in still other instances the nucleus was not visible. The trypanosome stained usually a deep blue color

with the Hastings' stain. The blepharoplast occupied the same relative position as in the trypanosome of the calf. The undulating membrane was well developed, but the free flagellum was short, measuring from 7.2μ to 13.5μ; occasionally no flagellum was seen.

In spite of the differences in size and staining properties, the trypanosomes in the 2 instances are identical, the chronic infection in the cattle with slow multiplication of the trypanosome yielding large individuals, while the acute infection in the calf with rapid multiplication gave rise to smaller organisms. These observations suggest that possibly Tr. ingens is identical with Tr. theileri, since the staining characteristics of this organism closely resemble those of the trypanosome in our cattle; however, the maximum measurements of Tr. ingens are decidedly greater than ours.

The following animals were inoculated with young cultures of the trypanosome: a guinea-pig, a monkey, a pigeon, and a chicken. Repeated examinations of the blood of these animals during the succeeding weeks were negative for trypanosomes except in the case of the pigeon. A large trypanosome was seen in a cover-slip preparation of the fresh blood of this bird on the 11th day after inoculation, but none were found in stained films. The concentration method could not be applied to this blood, because the red cells of the pigeon are nucleated and the nuclei are not disssolved by distilled water. Cultures from the pigeon's blood failed to show trypanosomes; hence it seems probable that the trypanosome seen in the cover-slip preparation was Tr. columbae and not the cattle trypanosome with which the bird had been inoculated.

In order to eliminate the possibility of a loss of virulence of the trypanosome in the culture, the blood of cattle known to harbor the trypanosome was injected subcutaneously into the following animals: 4 guinea-pigs, 1 chicken, 2 pigeons, 1 monkey, 3 opossums, 2 puppies, 1 goat and 1 mule. One trypanosome was seen in a cover-slip preparation of blood of 1 of the pigeons on the 5th day after inoculation, but this was in all probability Tr. columbae. The results were entirely negative in the other instances; the animals showed no signs of illness and trypanosomes were not demonstrated in their blood. For each group of the animals mentioned that were inoculated at the same time, the mixed, defibrinated blood of 2 or 3 steers was used.

In contrast to these results the inoculation of a young calf yielded particularly interesting results and the protocol of this experiment will be given in detail.

A heifer calf about 4 months old from Corozal Farm was inoculated with smallpox vaccine on June 27, 1917, and the vaccine was collected on July 3, 1917.

July 8, 1917.—The calf was bled from the jugular vein and cultures were made in broth. No trypanosomes ever appeared in these cultures.

July 9.—Ten c.c. of the mixed defibrinated blood of 2 steers was inoculated into the calf subcutaneously. Trypanosomes were demonstrated in the blood of the 2 steers. Smears from the calf's blood remained negative.

July 25.—The calf was bled from the jugular vein and the blood was cultured. The cultures remained negative for trypanosomes. Smears from the calf's blood taken at intervals were negative.

Aug. 4.—The calf was given 32 c.c. of the mixed, defibrinated blood of 3 steers known to harbor the trypanosome.

Aug. 16.—The blood of the calf contains numerous trypanosomes. Bled from the jugular vein. Cultures in broth at 24-26 C. were positive and showed the same marked changes in the morphology of the trypanosome that were previously encountered in cultures from steers. The calf's blood inoculated into a puppy, a monkey, a goat and a mule yielded only negative results. The defibrinated blood treated with an equal volume of distilled water and centrifugated strikingly confirmed the value of the concentration method we had been using for the steer's blood. In stained smears of the calf's blood a trypanosome was encountered only after passing over a dozen or more fields with the oil-immersion lens; after concentration there were from 1-10 trypanosomes to a field.

Aug. 20.—Blood smears positive for trypanosomes.

Aug. 24.—Blood smears positive for trypanosomes.

Aug. 27.—Blood smears negative for trypanosomes.

Aug. 31.—Blood smears negative for trypanosomes.

The trypanosome of Panamanian cattle agrees with the description of Tr. theileri (1) in size, (2) in morphology, (3) in being readily cultivated in broth at 26 C., and (4) in being unable to develop in other animals than cattle. We have therefore concluded that it is identical with this trypanosome.

Tr. theileri was discovered in the Transvaal in 1903. It was soon afterwards found in other parts of Africa, in India and Eastern Asia. It was first cultivated by Miyajima in Japan and later by Martini in the Philippine Islands, but these authors did not recognize the trypanosomes which they studied as Tr. theileri. Martini thought he had to do with a new species partly because the distal end of the flagellum of the trypanosome which he cultivated was clubbed. This feature is quite common in our cultures, but does not occur in the trypanosomes stained directly in the blood. It is obviously dangerous to attempt to describe trypanosomes from cultures alone. Crawley obtained cultures of a large trypanosome (Tr. americanum) in the United States of America. Tr. theileri has been obtained in cultures in Germany, England, France, Denmark, Sweden, Greece, Algiers, Tunis and Uruguay. It is regarded by almost all writers as being nonpathogenic.

SUMMARY

Tr. theileri occurs in a large percentage of beef cattle in Panama.

It is present in such small numbers in the peripheral blood, that stained smears are uniformly negative.

After defibrinating the blood, treating with an equal volume of distilled water and centrifugating, we find it almost always in smears from the sediment.

Filaria are also readily demonstrated in the blood of Panamanian cattle by the same procedure, although they are very rarely found in ordinary blood films.

The trypanosome is readily cultivated in broth at 24-26 C., but undergoes marked changes in morphology in the culture. Inoculation into other species of animals than cattle yielded negative results.

PIROPLASMOSIS OF CATTLE IN PANAMA

VALUE OF THE BRAIN FILM IN DIAGNOSIS

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The first important observation relating to piroplasmosis of cattle appears to have been made by Reverly[1] in 1881. He pointed out that the disease was spread by a tick, Boophilus bovis. Babes is credited with having been first to give it microscopic study and to have claimed that it was caused by a parasite (hematococcus bovis) in the red blood corpuscles. He also learned that the disease could be transmitted from sick to healthy animals by means of direct inoculation with blood or kidney substance from an affected animal.

Smith and Kilborne[2] gave the disease its first exhaustive study. The nature, cause, agent and prevention of the disease were so well elucidated by them that but little has been added since save as regards its geographic distribution and the evidence indicating that more than one species of the parasite exist in different parts of the world.

DEMONSTRATION OF PIROPLASMOSIS IN PANAMA CATTLE

"Southern cattle fever" or a disease quite similar to it, has been found in Finland, Norway, Italy, Turkey, Germany, France, Holland, Hungary, England, Portugal, Africa, India, Java, The Philippines, Australia, Mexico, South America (Brazil, Uruguay, Argentina, and Chili), Cuba, and Porto Rico. It would seem reasonable, therefore, to suppose that Panama and other Central American countries should have the disease.

The early construction period of the Panama Canal was not associated with the use of many domestic animals. The meat and dairy products used were imported chiefly from the United States or purchased from the local markets. As the work progressed, draft animals were needed and a comparatively large number of mules and horses were imported. During this period a few small herds of native cattle, hogs, horses, and goats were scattered over the Canal Zone.

Not long after the importation of the mules and horses some of them came in contact with native animals and S. T. Darling,[3] then the director of the Board of Health Laboratory, discovered murrina, a fatal trypanosomal disease of horses and mules. A little later, he also encountered equine piroplasmosis.[4]

[1] Hutyra and Marek: Pathology and Therapeutics of the Diseases of Domestic Animals, 1916, 1, p. 764.

[2] U. S. Dept. of Agriculture, Bureau of Animal Industry, Bull. 1, 1889.

[3] Jour. Infect. Dis., 1911, 8, p. 467.

[4] Ibid., 1913, 13, p. 197.

Near the middle of the construction period, Ancon hospital organized a dairy and stocked it with animals from the United States, chiefly from the gulf coast region. To this dairy were added some imported hogs and large parks of chickens. This enterprise grew to such proportions that it soon required more space and it was removed to a nearby village where it is now known as the Corozal hospital farm. A few cases of tuberculosis were found in the imported cattle, a condition that is almost never found in the native cattle. Acute infectious abortion and other conditions, common elsewhere, were at times encountered among these animals. Hog cholera also made its appearance. It was noted that the calf mortality was higher than is usually expected in well cared for stock. Clinical and post-mortem observation failed to explain this death among the calves and although suspicion was directed at piroplasmosis the presence of the disease could not be established.

The development of the Ancon hospital dairy stimulated local cattle owners in Panama City to more intensive dairy farming and they imported Holstein and Jersey cattle to be crossed with native stock in order to increase the output of dairy products. These animals soon became sick and many of them died. Piroplasmosis was suspected again, but this diagnosis could not be established by microscopic examination. In an outbreak of acute disease in a herd of Holstein cattle, prolonged search was made of the blood films from all the cattle, native and imported, of the dairy in question and Dr. Darling made a necropsy on a Holstein animal. The clinical picture of the disease in the imported animals was that of "southern cattle fever" and in his report (January, 1915) Dr. Darling so indicated, although the only bodies found were some "anaplasma-like" bodies in the blood films of the animal examined after death.

At the close of the construction period of the canal, a supply department was organized and one of the subdivisions of this service is known as the department of cattle industry. Large pastures have been cleared along the course of the canal for the purpose of receiving imported cattle and hogs. Chicken farms were also organized. The imported stock after being fattened in the pasture are slaughtered and furnish the meat supply. Dr. W. J. Taylor of the veterinary staff and Major (now Colonel) F. F. Russel, U. S. Army, the director of the Board of Health laboratory, began to suspect certain cattle in one of these pastures of being ill with "southern cattle fever" although they could not find parasites in the blood films. Nov. 22, 1916, two calves from the Corozal hospital farm in a moribund condition were killed and examined with the following results:

CALF 1220.—Emaciated, yellow heifer, about 10 months old, weight 200 lbs. The hide was tick infested. There was extreme degree of anemia but no sign of jaundice or of hemoglobinuria. An acute fibrino-purulent peritonitis was present in the upper half of the cavity and in the upper end of the small bowel several large patches of a fibrinous exudate were found. No marked change in the spleen. Parenchymatous degeneration in the liver, kidneys, and heart muscle, but no petechial hemorrhages in any of the serous membranes. Films were prepared from the various organs and the blood, and stained with Hastings' modification of the Romanowsky method. A scant number of Babesia bigemina were found chiefly in the bone marrow of the rib and the crushed gray matter of the brain cortex.

The intestinal and peritoneal lesions may have had much to do with the death of this animal and the piroplasmosis may have been only of the chronic type.

Calf 1221.—A small heifer, weighing 35 lbs., about 6 months old. The hide was tick infested; the animal was extremely emaciated, very anemic, but there was no jaundice or hemoglobinuria. All serous membranes contained areas of ecchymosis, and the acute parenchymatous degeneration was extreme. The spleen was enlarged, the capsule tense, the pulp soft, oozing, and purple. The brain did not show any sign of lesion except an intense degree of congestion.

Films from the various organs in this case all revealed Babesia bigemina, but the films showing the greatest abundance were those from the gray matter of the brain cortex and ganglionic matter at the base. These preparations showed capillaries literally stuffed with paired, pyriform bodies. Some of these films were sent to Dr. Theobald Smith who confirmed the belief that the parasites were Babesia bigemina.

Four weak, emaciated, tick infested calves soon arrived from the Miraflores pasture of the supply department and these were kept under observation from a few days to 3 weeks. They had fever but did not show the typical curve of the acute disease. Blood films were examined each morning and evening and in one case revealed a few parasites in the peripheral blood as well as in preparations made from a blood engorged female tick. An effort to further utilize this method of examination did not prove practical. No jaundice or "red water" was noted in these calves while under observation, but they may have had periods of hemoglobinuria since it was not possible to observe all voidings. The reduction in the hemoglobinuria was marked since the average reading was 50%. The ultimate diagnosis of piroplasmosis was made at necropsy. All very sick animals in this particular pasture were now located and kept out of the herd. A tick eradication was then instituted along the lines employed in the gulf coast region of the United States. Ticks from some of the animals were studied by L. H. Dunn, entomologist, who reported them to be Margaropus annulatus australis.

In the meantime I examined 25 more animals that had been taken away from the herd, all calves from 6-10 months of age except 2 old native cows. In all 29 cattle were examined and the film study except in 4 cases included only those prepared from the brain, spleen, marrow, and blood.

The following results were obtained as to the presence of Babesia bigemina:

	Positive	Negative
Brain films	29	0
Marrow films, rib	10	19
Spleen films	8	21
Blood films	3	26

For some reason the parasites were easier to find or were more numerous in the brain capillaries than in the other 3 preparations. The constant presence of the parasites in the brain capillaries in this disease is even more striking than in the case of the parasite of malaria in the human being.

The cattle thus far in question were born on the Isthmus, and most of them were the calves of native cows bred to an imported bull. Since birth they had been exposed to tick infestation. The universal presence of the parasite in these animals indicated that a latent type of the disease might be common among the native cattle outside the Canal Zone.

Through the kindness of Dr. Baltasar Bosch, city veterinarian in Panama, and of Dr. W. J. Taylor, veterinarian in charge of the cattle industry in the Canal Zone. I was permitted to study an unselected series of prime beef animals immediately after they were killed. Further experience with films of various tissue, including the heart, kidney, and liver, led me to continue the use of brain films.

Three or four blows with an ax, hatchet, or machete just behind the horns will expose the brain, and as a rule the dura and the brain are not badly lacerated. A piece of the gray matter of the brain cortex about the size of a split pea was selected, placed on a glass microscope slide near its middle, another slide being used to crush it so that a film extends over half the slide. By increasing the pressure as one drags the material toward the ends of the slide it is possible to leave a film that is thick at the middle of the slide and thin at the end. At some place in the preparation networks of blood-filled capillaries will be found. The film is quickly dried and then stained as soon as possible in some polychrome blood stain. When ready for examination use low magnification to find some well defined thin areas containing capillaries, and then a high magnification. The capillaries can then be traced for blood cells containing parasites. Many times the first field of capillaries examined in the slaughter-house revealed one or more parasites. The average time spent in searching in a particular case was 3 minutes; although some of the cases required 20-30 minutes.

The beef cattle of Panama came from all parts of the cattle region of the Republic. They were examined in lots of 20-25 just in the order that the authorities chose to kill them without regard to age or degree of tick infestation. Every animal of the 125 examined showed Babesia bigemina and in spite of a rather fair degree of infection in some offered no clinical sign of the disease.

The supply department imports a similar class of animals from Colombia which are slaughtered at Cristobal. An examination of 150 of these showed that 90% were carriers of the same parasite.

Other opportunities have been taken to examine cattle of all types in outlying regions as well as in the basin of the Chagres river as far up the river as inhabitants are to be found. In all localities the disease was present. Native cattle suffering serious injury, long periods of starvation, etc., develop piroplasmosis, at least their examination showed an abundance of parasites.

It appears from these results that almost all the cattle of Panama and Colombia must be considered as carriers of the parasites of piroplasmosis. Since no protective measures are employed against the disease and since the tick in this locality continues an almost uninterrupted cycle of development throughout the year, all cattle born here are exposed to the infection from the time of their birth on. It is well known that calves survive the disease better than the fullgrown cattle that have not had the disease in their early life, nevertheless, it is possible that this disease partially explains the high death rate in calves in this country. The animals that do survive the calf period certainly acquire an immunity that protects them from future attacks provided injury, starvation, or some intercurrent disease does not undermine

the vitality. The importation of nonimmune cattle, when not followed
by protective measures, is surely followed by considerable loss.
Generally speaking, the native cattle (although hardy animals) do not
fill out into the fat sleek animals commonly found outside a tick belt.

PECULIARITIES OF PIROPLASMOSIS OF PANAMA CATTLE

The acute cases found in Panama, as a rule, occur in nomimmune
imported cattle. Many of the fullgrown animals of this class are lost,
but a fair percentage of the calves survive. The clinical picture of the
acute disease is like that described by Smith and Kilborne.[2] Native
animals, as already mentioned, suffer acute attacks when they are sub-
jected to starvation, injury, or an association of diseases. In the
chronic form the animal appears wasted, anemic, and lazy, with a tick-
infested hide. Jaundice and hemoglobinuria are not often noted, and
the temperature curve is not characteristic. Carriers of the parasite
may not show a single sign of the disease except possibly the fact that
they do not become large fat animals.

In acute cases there is often a lemon colored conjunctiva and oral
mucous membrane. Punctate hemorrhages in these regions are not
infrequent. The number of ticks present may not seem noteworthy.
Cattle dying of the more chronic type are often heavily infested with
ticks and may show extreme wasting and anemia.

The head and neck often offer little that helps to differentiate the
disease. There is no sign of acute inflammation. Acute cases reveal
intense congestion of the meninges and gray matter often with miliary
and larger hemorrhages. In chronic cases the brain is usually pale,
with increase in fluid. The most important feature is finding the para-
sites in the brain capillaries, in acute as well as chronic types of the
disease. My experience does not indicate the meninges and choroid
plexus to be as satisfactory as the brain film already mentioned.

It appears from the report of Smith and Kilborne[2] that the brain
was examined in only 4 of their cases, and here they found great
numbers of parasites in the pia, choroid plexus and the brain substance.
Since these were probably acute cases, this organ did not impress them
as a suitable one for routine examination.

Punctate hemorrhages in the lungs may be found in acute cases and fre-
quently areas of pneumonia are present and are covered on the pleural sur-
face by a granular exudate. Chronic cases usually show pale somewhat
edematous lungs. In acute cases there may be extensive areas of ecchymosis

in the myocardium, pericardium, and endocardium. At times there is a yellow tint in the endocardium. Chronic cases as a rule show an increase of fluid in the pericardium, and a pale soft heart muscle. Almost all animals have sarcosporidia, the sporozoits being found in large numbers in films from the heart muscle. The abdominal wall is usually edematous, with smaller and larger hemorrhages; minute hemorrhages occur frequently in the peritoneal coat of the stomach and intestines. In chronic cases there may be some effusion in the peritoneal cavity.

In acute cases there is an enormous enlargement of the spleen with a tight, smooth capsule, sprinkled with punctate hemorrhages. The color of the capsule is darker than normal. The pulp is purple, semidiffluent, and oozes so much that the malpighian bodies are hidden. It is difficult to say anything definite about the spleen in chronic cases because all native cattle harbor several types of latent infections that may play some rôle in modifying the spleen. The organ usually is a little enlarged, the consistency increased, the capsule is thicker than normal and almost white in color, wrinkling easily.

In acute cases there is marked enlargement of the liver, which has a mottled brown and yellow color, the margin rounded, pressure necrosis being present at times. The bile is usually thick, the gallbladder frequently distended. Chronic cases present a pale liver without noticeable increase in size.

In acute cases the kidney is greatly swollen, mahogany colored, sometimes sprinkled with small hemorrhages, the cut surface of the cortex showing brown and yellow striations. Hemoglobinuria is occasionally present; albumin is present in large amounts. Chronic cases have a pale yellow kidney, sometimes enlarged; there may or may not be albuminuria, and hemoglobinuria is seldom seen.

Intestinal parasites and inflammatory processes are so common among cattle that it is difficult to determine the influence of this disease on the variety of lesions found. In calves only congestion and minute hemorrhages in the serous coat have been found and it is probable that nothing more than this is caused by the disease in older cattle.

There is a marked reduction in the hemoglobin in both the acute and chronic forms of the disease. In other parts of the world from 1-10% of the red corpuscles in the peripheral blood are said to be infected. The puzzling feature of the disease in Panama is that in never in more than 8 or 10 cases have I been able to find the parasite in the peripheral blood, even in acute cases. I attribute our delayed identification of the disease in this region to the extreme scarcity of the parasite in the peripheral blood and to our failure to open the cranial cavity in animals that died of the disease. Concerning the distribution of the parasites in the body Smith and Kilbourne[2] report as follows: "They are very abundant as determined thus far, in the capillary blood of the heart muscle, but quite rare in the skeletal muscles. Of the internal organs the kidneys usually contain the largest numbers, not infrequently from 50-80% of all corpuscles are infected. Next come the liver and spleen. In spite of the fact that the spleen is loaded with red corpuscles by several times its own weight, rarely

more than one-tenth contain parasites. Infected corpuscles have been found in great abundance in the capillaries of the choroid plexus and in the vessels of the pia and the brain substance. They have also been found in the capillaries of the intestinal mucosa.

They further report that: "The brain was removed in a small number of cases and carefully examined, but no lesions which can be regarded as peculiar to or characteristic of the disease were observed. It may be said, in general, that the brain shared the general tendency toward the injection of the capillary system. The gray matter of the cerebrum and especially of the cerebellum appeared of a more pinkish color." It would appear that the brain was not given that routine film study that was accorded the heart muscle, kidney, liver, etc.

Local experience with acute cases in imported stock (initial attack) largely confirm the statements just quoted, but notwithstanding the fact that an abundance of parasites can be found in films prepared from the heart and kidney, the brain has always revealed a greater abundance, and when chronic cases and carriers are to be examined at necropsy or in the abattoirs the system of brain capillaries take precedence over all others. It is reasonable to suppose that organs with intricate capillary systems favor the presence of such parasites, but the capillaries in the kidney, heart muscle, liver, spleen, etc., do not lend themselves readily to the preparation of films free of débris and which offer a sufficient number of intact small capillaries. The brain cortex is rich in small capillaries, and by selecting a bit about half the size of a pea, one can press it between 2 glass slides and drag them apart with increasing pressure as the ends are approached so that a thinner and thinner film is made. Now somewhere in such film, usually about the middle, an abundant network of capillaries is found. The slide should be dried quickly and stained at once if possible. Any modification of the polychrome used as customary for staining blood will answer. Russell's[5] method is highly recommended if a large number are to be examined, being a good field method since it saves time and stain, and wide-mouthed bottles serve for staining jars. In a brain film practically all the blood cells will be held inside the capillaries which thus isolate the blood from confusing elements as in other tissue films. The capillary wall does not prevent the penetration of the stain. The number and small size of the cerebral capillaries form a good seine in which infected blood cells are caught. Acute cases will show

[5] Jour. Am. Med. Assn., 1915, 64, p. 2131.

all capillaries in the brain film full of infected red cells. Chronic
cases and especially carriers may require the tracing of several capillary
networks. In chronic cases and carriers, such as steers, I have scores
of times readily found the parasites in the brain film when the films
from all other organs were negative after a long search.

The common type found in the brain is the pair of pyriform bodies
lying so that their sharp ends approximate or even appear to touch.
The two bodies usually form a rather acute angle, but the degree of
this angle may vary greatly, in fact, many times they appear almost in
a straight line. The two bodies may vary in both size and shape to
some extent. Some cases show a modification of the pyriform shape
that closely approaches a diamond or kite shape. In such cases, how-
ever, the large end of the body has a much more blunt point than the
small end. The polychrome stain gives the parasite a blue color and
the center of the large end is lighter in color than the rest. A well cir-
cumscribed red dot is frequently found in the large end of the paired
bodies and it is somewhat laterally located. Frequently this red
material is not collected into a discrete body, but may appear as
granules arranged in a diffuse manner chiefly in the large end of the
parasite.

Films that have been dried slowly before staining or that have been
prepared several hours post mortem will show the parasites staining a
solid blue color and contracted in appearance. Short blunt pyriform
pairs and even larger diplococcal bodies are common.

A greater variation in the size and morphology of the parasite has
been found in films from the marrow of the rib. Here also the com-
mon type is the paired pyriform body, but the variation in the size of
the 2 bodies, in the angles formed by them, and even in the relation of
the 2 bodies to each other in the cell is quite remarkable. Sometimes
they are parallel, sometimes in reverse directions, and at other times
they occupy totally different parts of the cell.

Single pyriform types and comparatively large irregular round
forms that simulate the parasite of malaria are infrequently seen.

The type in the peripheral blood film of a few acute cases were
single pyriform bodies larger than one of the paired forms found in
the brain.

So few "anaplasma-like" bodies were found in the blood films and
their position was so inconstant that it is unsafe to say that the bodies
really represent parasites.

The average size of the typical paired pyriform bodies found in an acute case in the brain measured 3.6 by 1.8μ.

The following animals were inoculated with the defibrinated blood from beef cattle known to be *carriers* of Babesia bigemina: 4 guinea-pigs, 1 chicken, 2 pigeons, 1 monkey, 3 opossums, 2 young dogs, 1 mule, 1 goat, and 2 nonimmune calves. The calves developed the disease, but no results were obtained in any of the other animals.

One faces many difficulties in the study of a particular disease in native cattle because these animals are the hosts to so many parasites. The skin, muscles, blood, respiratory tract and alimentary tract all have their parasites. In making observations on piroplasmosis it has been found that sarcosporidiosis, filariasis, trypanosomiasis were all about as prevalent as piroplasmosis. Dr. Oscar Teague and the author have succeeded in almost every attempt to grow a large trypanosome from the slaughter-house animals. I have found a spirochete in the blood film of one animal and Dr. Teague has grown one from another animal. They are probably S. theileri.

There is a striking absence of tuberculosis and of the cysticercus in the native cattle.

Anthrax was noted by Darling and Bates in the early years, but the first real epidemic occurred recently and was placed under control by the efforts of Major (now Col.) Russel and Dr. W. J. Taylor. Actinomycosis has been seen once in native cattle.

While visiting some outlying districts and the basin of the Chagres River I had the opportunity to examine wild animals, among them 10 deer; 4 of the deer contained parasites that seemed to be identical with the piroplasma in cattle.

About 24 imported hunting dogs have been examined and 3 of them have shown Piroplasma canis.

Panama is a tropical country and therefore favors tick development without much seasonal interruption. Native cattle run at large and are at no time stable fed or protected unless used for dairy purposes or as draft animals. Preventive measures now in use at the Corozal hospital and farm and the Miraflores pasture of the supply department show that piroplasmosis and tick infestation can be controlled to a large extent even in Panama. Although native animals are fairly secure in their acquired immunity, it will be interesting to see what results will be obtained when tick eradication is undertaken. Certainly it seems reasonable to expect a drop in calf mortality, an increase in the weight

of beef animals, and a greater output in dairy products. Prevention and protective measures have proved their value in other places.

SUMMARY

Piroplasmosis of cattle is present and practically speaking universal in Panama.

Nonimmune cattle when imported into Panama contract the disease and many of them die.

A positive antemortem diagnosis of piroplasmosis in this locality is extremely difficult on account of the scarcity of the parasites in the peripheral blood.

The examination of the blood in films of the gray matter of the brain makes the detection of the parasite at necropsy and in the abattoir comparatively easy.

In Panama piroplasmosis has been found in the horse, cattle, deer, and dog.

Investigations of native cattle led to the observation that they are hosts in almost every instance to Babesia bigemina, filaria, sarcosporidia, and a large trypanosome, probably of a harmless type. Two cattle revealed a spirochete, probably S. theileri.

STUDIES IN EPIDEMIC POLIOMYELITIS

I. THE ISOLATION AND CULTIVATION OF THE GLOBOID BODIES

PLATE 5

George D. Heist, Myer Solis-Cohen, and John A. Kolmer

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In 1913, Flexner and Noguchi isolated a minute, anaerobic micro-organism from the nervous tissues of human beings and monkeys dying of acute poliomyelitis. They were uncertain regarding the nature of these organisms and referred to them as "globoid bodies." They cultivated the micro-organism with success, inoculated pure cultures into the brains of monkeys and produced clinical and anatomic poliomyelitis; from the nervous tissues and blood of the infected monkeys the globoid bodies were recovered in pure culture.

In this paper we wish to record the isolation from human and monkey poliomyelitic material of 4 different strains of an organism which morphologically and culturally agrees in every particular with the descriptions of the globoid bodies of Flexner and his associates.

In January, 1917, we began the work on 5 lots of glycerolated human poliomyelitic nerve tissue, obtained during the epidemic in the summer of 1916, at the Philadelphia Hospital for Contagious Diseases. The tissues had been in 50% glycerol for 5 months. With the writings of Flexner and his associates before us, we proceeded to follow the methods described by them with the utmost exactness. The tissue was emulsified, filtered through paper and some specimens were passed through Chamberland and Reichel filters. The filtrates were distributed among tubes of ascitic fluid to which fragments of sterile rabbit kidney had been added. Two c.c. of filtrate was placed in each tube. The fluid was overlaid with a layer of sterile paraffin oil approximately 2 inches deep and the tubes placed in the incubator at 37 C. As means for producing an atmosphere of hydrogen were not available at this time, these tubes were not placed in an anaerobic jar. Within a few days, a few tubes became clouded and were found to contain gross contaminations and were discarded. The rest of the tubes remained perfectly clear. At the end of a month 1 tube inoculated with filtrate of a brain and in 1 inoculated with filtrate of a spinal cord a faint haze was detected about the kidney fragment. The haze slowly

grew up the tube to a point 2 cm. above the kidney and stopped abruptly, the fluid above remaining quite clear. A drop of the clouded fluid was withdrawn with a capillary pipet, and stained with Giemsa as described by Flexner and Noguchi. The films showed a few small, violet colored masses which, while they suggested groups of minute cocci, lacked any very distinct morphologic characteristics. No similar masses could be found in control tubes. Other tubes of kidney-ascitic fluid were inoculated from the original two. In 5 days these second tubes showed a faint haziness around the kidney and again smears showed the violet clusters which could not be positively identified as organisms. In addition to these violet masses a few large round, single and paired cocci were found which stained a deep blue. Nothing in the nature of transition forms between the two types of bodies could be observed. The clusters appeared to be made up of very small bodies, all of the same size and of a uniform violet color, whereas the cocci were large and distinctly blue. A third generation of tubes, planted from the second, clouded up uniformly to the oil after 24 hours in the incubator and were found to be filled with large, short chain streptococci. The violet clusters did not reappear. The large streptococci when transferred to broth and agar slants grew readily aerobically and, by methods detailed elsewhere by two of us, were proved to be identical with streptococci very commonly found in many localities associated with poliomyelitis. By no methods of aerobic or anaerobic cultivation we could devise were we able to force this large, short-chain streptococcus to resemble in any particular the cluster of very minute violet bodies we had seen in the first and second generation. The streptococci remained streptococci. We filtered cultures of these streptococci through the candles used originally for the brain and cord emulsion. A few of the streptococci evidently passed through; for some subcultures of the filtrates clouded up and some did not. It seemed to us probable that two distinct organisms had been present in the poliomyelitic tissue and had passed through the Chamberland and Reichel filters. The ones which were seen in violet masses in smears from the original tubes had grown well under the anaerobic conditions, while the large blue cocci apparently had been unable to multiply until oxygen had been carried down into the fluid by the pipets used for drawing off portions for examination and transplanting, and by shaking, unavoidable in such manipulations. With this possibility in mind we returned to the original tubes. Here, too, the streptococci had now grown abundantly and clouded the ascitic fluid up to the oil. We diluted these original tubes slightly with normal salt solution and passed them through a Mandler filter. The Mandler, a diatomaceous earth filter of American manufacture, is graded by immersing the candle in water for 24 hours and then determining the pressure necessary to force air through the pores while it is still under water. The one we used withstood 11 lbs. of pressure. Tubes of kidney-ascitic fluid were inoculated with 2 c.c. of these second filtrates, covered with a layer of oil and placed in a tall museum jar in the lid of which holes had been drilled and rubber tubes attached. The lid was sealed with vaselin and clamped down and hydrogen from a tank run in until pure hydrogen escaped from the outlet. While this undoubtedly did not remove all the oxygen it removed a large part of it. The tubes remained clear for two weeks. At the end of that time when they were carefully examined against a black background it was possible to distinguish a very faint haziness which stopped abruptly 1 or 2 cm. below the oil. The column of haze was thus considerably higher than when the hydrogen atmosphere had not been used. Smears stained as before with Giemsa showed very little. On an entire slide but 1 or 2

violet clusters, lacking definite morphology, could be found. The large blue cocci were not present nor did they again appear in any later subculture. When the fluid was examined under a dark field apparatus nothing resembling an organism could be seen among the hosts of dancing granules. It was not until we resorted to the examination of a simple hanging drop, using the oil immersion objective, that we saw anything that could be said to have a distinct organized morphology. By making a hanging drop with fluid drawn from around the rabbit kidney, reducing the illumination by lowering the Abbé condenser, and carefully focusing up and down in the drop certain tiny globular bodies could be seen in the fluid drawn from the inoculated tubes which were not found in fluid drawn from the control tubes. These bodies were exceedingly minute. Pneumococci added to the drop for comparison seemed gigantic beside them. After 4 or 5 days' incubation, pairs and threes of the bodies could be found— never many; several fields might be examined and only one or two groups seen. In fluid from both inoculated and uninoculated tubes tiny single bodies showing active brownian movements could be seen, but the grouping in pairs, chains and, later, clusters was characteristic of the inoculated tubes. In fluid from tubes 7-10 days old short chains of 10 or 12 globules were seen. These chains were often tightly coiled up and the organisms were of such size that the entire chain might have been enclosed in the capsule of a pair of pneumococci. Still later tiny clusters with outgrowing chains could be found. At that time several such clusters might be found in a field. Examining our cultures in this manner in the hanging drop we were able to watch the slow development of the organism. When the organisms were fairly abundant, a new tube was inoculated with about 1 c.c. and the process followed in the next generation. One of our strains, No. 2b, is now in the tenth generation. Six months were required to accomplish this number of transfers. In the later generations the organisms multiplied more rapidly and with more certainty. Of all the tubes used in the 10 transfers it was only the occasional one that showed a perceptible haze on inspection. The haze was recognizable owing to the fact that it ended some distance below the oil with a sharp line of demarcation: as soon as this line was broken by the pipet or by slight shaking in handling, the haze would become distributed throughout the tube and no longer recognizable. Tubes of medium prepared in exactly the same manner would differ slightly in their optical density. The haze due to the globular organisms was so slight that a control tube might look more clouded than the inoculated tube, but when comparison could be made between the upper and lower portions of the same column the difference could be recognized.

When the ninth subculture had grown well, tubes of semi-solid medium were prepared by first inoculating a tube of kidney-ascitic fluid (8 c.c. of fluid) as usual and then adding 5 c.c. of 2% agar, melted and cooled to 40 C. In this medium a faint haziness appeared which ended abruptly a short distance below the oil. This cloud remained visible, as the solidity of the medium prevented its being distributed throughout the tube.

During the first six months in only one or two instances did we succeed in staining the organisms satisfactorily. Smears made from tubes in which numbers of the globoid bodies could be seen in the hanging drop, and stained according to the method described by Flexner and Noguchi, either showed no organisms whatever or a very few faintly stained dots hardly to be distinguished in the deeply stained background. Before we adopted the hanging-drop method of examination of our cultures many tubes which remained clear and in which no staining organisms were found were pronounced sterile. It now seems probable that many of these tubes contained the globoid bodies but that they

were overlooked. Later we found a method whereby the globoid bodies could be stained with ease. A subculture from the third generation was made in a 100 c.c. Ehrlenmeyer flask of combined solid and fluid medium by the method described by Noguchi. This was placed in the anaerobic jar and the organisms allowed to grow for two months. At the end of that time the ascitic fluid and agar remained clear, but a slight whitish sediment covered the surface of the agar. In the hanging drop the fluid was found to be crowded with clusters and large masses of the globoid bodies. The fluid was drawn off and centrifugated at high speed for two hours and the sediment washed with physiologic sodium chlorid solution and again given a prolonged centrifugalization. The final sediment was taken up in salt solution and yielded 3 c.c. of a heavy white suspension.

When smears were made from these washed organisms it was found that they stained readily with many stains. The films were fixed in methyl alcohol for 1 hour or in formalin vapor for 5 minutes. Giemsa stain, diluted 1 : 10 with distilled water and applied for 1 or 2 hours, yielded the most satisfactory results. Carbolthionin and carbolfuchsin, diluted and used in the same manner, stained the washed organisms with great distinctness. Loeffler's methylene blue undiluted was taken up less readily. In our experience the washed organisms have been decolorized by Gram's method. The washed globoid bodies were readily phagocytized after having been digested with normal human serum.

Later we succeeded in staining the organism in the ascitic fluid by making a thin film and drying over-night in the incubator. The film was fixed in methyl alcohol for 1 hour and transferred to Giemsa, diluted 1 : 10 with distilled water, and the jar placed in the paraffin oven at 50 C. for 24 hours. But even by this method the globoid bodies were stained distinctly in only a small proportion of films and in many films were not stained at all. We have used two lots of Giemsa stain, both prepared from Grubler's powder, one more than a year old and one quite fresh. With the older stain the globoid bodies stained violet, while with the fresh stain the color was usually more bluish. Whether this difference was due to the age of the stain or to something in the preparation we are unable to say. Flexner and Noguchi state that the globoid bodies at times stain violet with Giemsa and at times bluish.

A stained film shows the minute organisms without particular arrangement. In the film made from a young growth the bodies are of the same size, and take the stain uniformly. In old cultures degeneration-forms appear. Some individuals in a group do not stain and some are slightly larger than the others. The largest barely approach the size of a small coccus.

In addition to the two original strains from human material we have isolated two other strains from monkeys with experimental poliomyelitis. Cultures were made of the brain and cord of a monkey which developed paralysis of one arm and showed typical poliomyelitic changes following the intracerebral injection of human virus sent to the University of Pennsylvania by Dr. Leake from a patient in West Virginia succumbing during the winter epidemic of 1917. Twenty tubes of kidney-ascitic fluid were inoculated with small pieces of brain and cord. A layer of oil was placed over the fluid but the tubes were not kept in an atmosphere of hydrogen. After 6 days' incubation 9 of the tubes were faintly and uniformly clouded up to the layer of oil. Subcultures into broth gave pure cultures of the same large, short-chain streptococcus we had found in the human poliomyelitic material. The balance of the tubes remained clear and repeated subcultures from them failed to show any growth. Thirty-three days after the cultures were made, a very faint haze was observed about the kidney

in two of the tubes which had remained clear up to that time and which had given repeated negative cultures in broth. The cloud did not ascend higher than 1 cm. above the kidney. Aerobic cultures from this cloud in blood broth and blood agar showed no growth. In the hanging drop, however, the globoid bodies were recognized in fluid from these 2 tubes. Numerous successful transfers of this strain into tubes of kidney-ascitic fluid have been effected. One tube was accidentally contaminated with a small gram-negative bacillus, probably B. proteus. The contaminating bacillus was removed by filtration through a Mandler filter and the globoid bodies grown from the filtrate.

Our second monkey strain was isolated from a piece of the spinal cord of a monkey showing typical poliomyelitis. We were furnished with the fragment of cord by the Pennsylvania State Department of Health Laboratory, through the courtesy of Drs. Samuel G. Dixon and James B. Rucker, Jr. It had been in glycerol for 5 months when used. The fragment, about 1 cm. long was emulsified and the emulsion passed through a Mandler filter and the filtrate planted in four tubes of kidney-ascitic fluid. These were overlaid with paraffin oil and placed in the hydrogen jar. In 6 weeks a faint haze was observed extending part way up one tube and in addition a fine granular precipitate was present. Hanging drop examiation showed a few globoid chains and clusters and a slender bacillus. The fluid in this tube was refiltered and the bacillus did not reappear in cultures from the filtrate, while the globoid bodies grew well.

In preparing 1 lot of kidney-ascitic fluid medium the fragments of rabbit kidney were placed alone in the test tubes and the basket containing them put on ice. Some weeks elapsed before the ascitic fluid was added, at which time the kidney fragments had become darkened. During the incubation to test the sterility of the lot, a small amount of hemoglobin diffused upward through the fluid. When these tubes were used for subcultures we were surprised to find that the globoid bodies grew more readily than in tubes in which fresh kidney fragments had been used. It seems possible that allowing the kidney fragments to age before adding the ascitic fluid may create a more favorable medium for the globoid bodies. Both of our successful isolations from monkey poliomyelitic material were obtained in tubes in which the kidney had been aged in this manner.

Successful isolations have been performed with two samples of ascitic fluid, and a third has proved successful in supporting growth of subcultures. It may be possible, therefore, that the variation among samples of ascitic fluid has less influence on the growth of the organism than has been supposed.

SUMMARY

A micro-organism has been isolated from human and monkey poliomyelitic nervous tissue which agrees morphologically and culturally in every particular with the globoid bodies first described by Flexner and Noguchi. Four different cultures have been obtained: two from human and two from monkey poliomyelitic tissue.

It has been observed that the macroscopic appearance of tubes of kidney-ascitic fluid medium which contain good growths of this organism differs from that of the uninoculated control tubes to such a slight degree that often inspection of the tube alone is of no value in determining the presence or absence of a growth.

The organism while in the ascitic fluid stains with such difficulty and in the earlier generations the minute globules are so few in number, that they may easily escape observation if stained smears are relied on to detect growth.

Careful examination in the hanging drop has proved a simple and rapid method of detecting the organism in fluid medium.

The organisms which stain with difficulty when in the ascitic fluid are stained with ease after they have been freed from the culture medium by washing in normal salt solution.

A more rigid anaerobiosis than can be obtained by the use of a layer of paraffin oil is advisable for the successful cultivation of this micro-organism.

The use of rabbit kidney which has been allowed to age before adding the ascitic fluid may be one factor in creating a successful artificial medium for the cultivation of the organism.

EXPLANATION OF PLATE 5

Fig. 1. Washed globoid bodies. Streptococcus pyogenes has been stained on the same slide to show comparative size. Giemsa stain. × 1200.

Fig. 2. Washed globoid bodies. Pneumococcus has been stained on the same slide to show comparative size. Giemsa stain. × 1200.

Figs. 3 and 4. Washed globoid bodies and Rosenow's streptococcus. Pure cultures of each organism were smeared and stained in the same slide for comparison. Large degeneration forms characteristic of old cultures of globoid bodies may be seen. The clumping is due partly to the salt solution used in washing. Giemsa stain. × 1200.

Fig. 5. Washed globoid bodies showing phagocytosis in vitro. Giemsa stain. × 1200.

PLATE 5
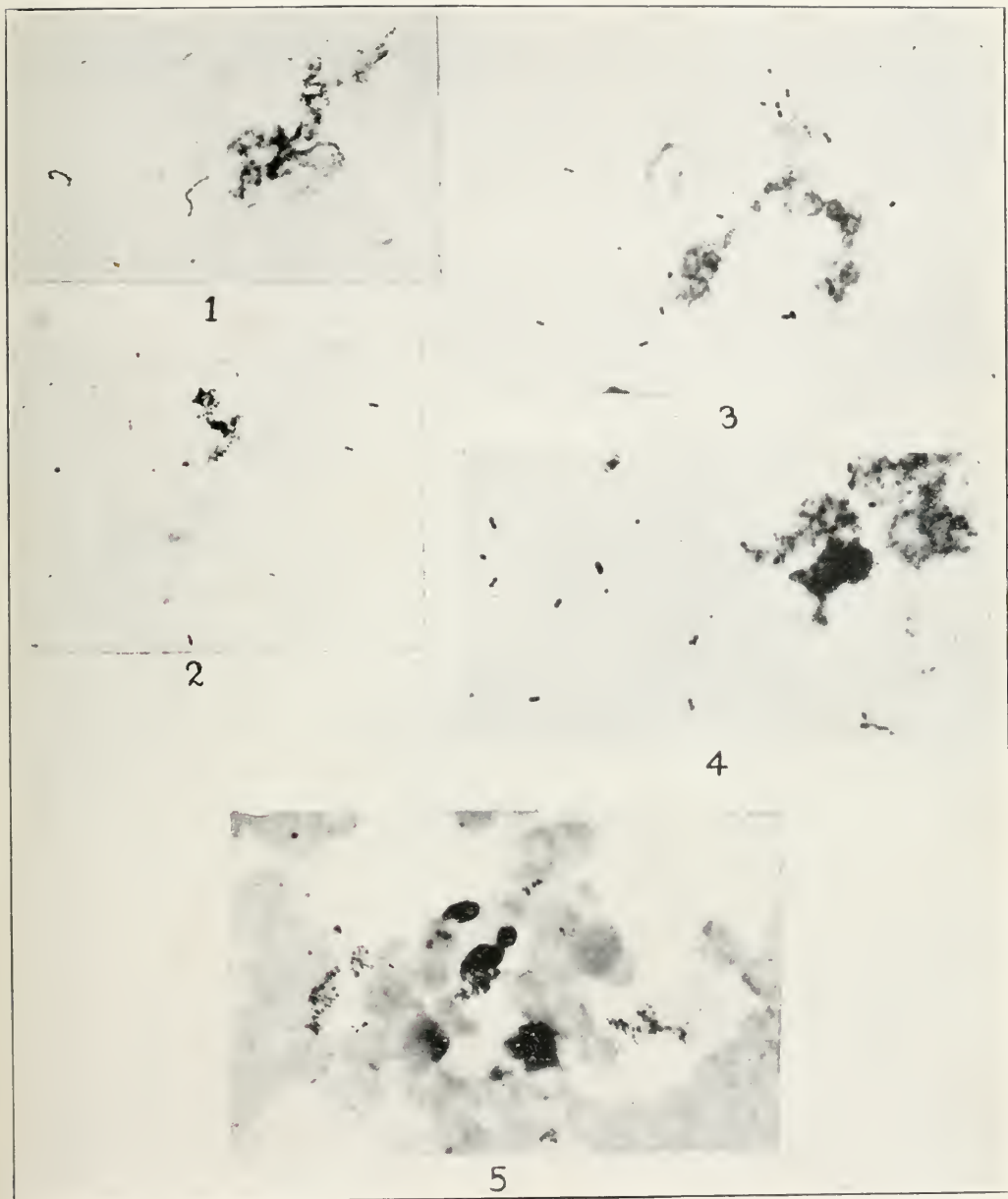
STUDIES IN EPIDEMIC POLIOMYELITIS

II. A STUDY OF THE OPSONIC ACTION OF POLIOMYELITIC SERUM ON VARIOUS ORGANISMS ISOLATED FROM FATAL CASES OF POLIOMYELITIS

Myer Solis-Cohen and George D. Heist

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In addition to the globoid bodies isolated in conjunction with John A. Kolmer, we have isolated from the same poliomyelitic material three strains of a rather large streptococcus occurring in short chains. These strains came from the nervous tissues of two patients dying of poliomyelitis in Philadelphia during the summer of 1916, and from the nervous tissue of a monkey that contracted poliomyelitis following the injection of virus from a human being dying of poliomyelitis in West Virginia during the winter of 1917. This streptococcus was distinct from the globoid bodies, having a different appearance in culture, hanging drop, and stained specimen. The streptococci grew abundantly aerobically, while the globoid bodies required strict exclusion of oxygen. The streptococci stained differently from the globoid bodies. The globoid bodies passed readily through a Mandler filter, whereas it was only occasionally that a growth was obtained from the filtrate of a pure culture of the streptococci.

Others also have isolated cocci from cases of acute anterior poliomyelitis, notably Mathers,[1] Rosenow[2] and his associates, Nuzum and Herzog.[3] At the Philadelphia Hospital for Contagious Diseases in September, 1916, Kolmer, Brown and Freese[4] isolated many strains of Streptococci and other bacteria from the brain, cord and internal organs of fatal cases.

We have studied a number of these organisms serologically to see if serum from patients with poliomyelitis would give a specific opsonic reaction with any of them that might point toward its presence at some time in the tissues of the patient.

[1] Jour. Infect. Dis., 1917, 20, p. 113.
[2] Jour. Am. Med. Assn., 1916, 67, p. 1202.
[3] Ibid., p. 1205.
[4] Jour. Exper. Med., 1917, 25, p. 789.

Types of Strains Employed

Rosenow supplied us with three strains of streptococci: No. 44, "the fourth subculture of a strain from a monkey having poliomyelitis following injection of a filtrate of a virus of a monkey that was paralyzed with a filtrate of a virus."

No. 49, "a third subculture from a Berkefeld filtrate of the cyst wall of a monkey injected with a culture that was isolated from the central nervous system of a monkey paralyzed with a filtrate of a virus."

No. 707, "a strain isolated from a single colony from the spinal cord of a case of poliomyelitis in man . . . now in the ninth subculture."

Kolmer, Brown and Freese supplied us with a Streptococcus 20, isolated from the pons of a patient dying on the seventh day, in addition to many strains of staphylococci, diphtheroids and gram-negative bacilli, all obtained from poliomyelitic material.

In making the opsonic tests the method of Wright[5] was followed minutely. Cultures for the work were made on solid mediums, washed off and suspended in 1% salt solution. Human leukocytes were used, obtained in every case from the same individual. These, too, were suspended in 1% salt solution. A 1% rather than normal salt solution was used because Wright found that in this slightly hypertonic solution spontaneous phagocytosis does not take place. In making the tests equal volumes of bacterial suspension, washed blood corpuscles and serum were mixed in capillary glass tubes and incubated in a horizontal position at 37 C. for 20 minutes. Smears were made and stained with dilute carbolthionin. The bacteria ingested in the polynuclear white blood corpuscles were counted until it was observed that further counting would not change the average.

Serum was obtained from acute cases of poliomyelitis at the Philadelphia Hospital for Contagious Diseases, and from convalescents at the Wynnefield Branch of the Children's Hospital and at the Orthopedic Dispensary of the Hospital of the University of Pennsylvania. Since November, 1916, when our work was begun, there have been very few acute cases in Philadelphia and only one seen by us as early as the eighth day. Most of the convalescents were paralyzed in the summer of 1916, a few being cases of longer standing. Blood was obtained from the finger and allowed to clot. The serum was not heated and was examined the day after collection, since serum retains its opsonizing power undiminished for two or three days.

As a control, serum from the same healthy individual was carried through with every series of tests. In addition, in many series control serum was obtained from adults and children, normal so far as any history of poliomyelitis was concerned. Such serum was either tested separately, or equal volumes of a number of normal serums were mixed together and a portion of the pool thus formed tested. With the strains we used, the average number of bacteria ingested varied but slightly with the different adult serums employed. With normal children the average count was usually a little below that of the adult. The serum of normal babies less than a week old when tested with Streptococcus 20 gave an average count invariably but one-half to two-thirds of that produced by adult serum. Thus opsonin for Streptococcus 20, with which most of this work has been done, was low at birth and increased slowly with age. We have followed strictly the method of Wright, who insists that each day's work must be controlled by one or more normal serums, the average ingest of which is taken at 1.0 in computing the opsonic index.

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[5] "Technique of the Teat and Capillary Glass Tube," London, 1912.

With most of the strains tested, including No. 49, poliomyelitic serum gave results which did not justify further investigation, and early in the work we eliminated all but three strains. These three were Nos. 44 and 707, isolated by Rosenow, and 20, isolated by Kolmer, which last we have found to be identical with the streptococcus isolated by us.

TABLE 1

The Opsonic Action of Human Immune Poliomyelitic Serum on Streptococcus 20

Age	Sex	Time Since Onset	Opsonic Index	Age	Sex	Time Since Onset	Opsonic Index
3 months	M	19 weeks	0.9	2 years	M	16 weeks	1.4
6 months	F	19 weeks	1.3	2 years	F	14 weeks	1.2
7 months	M	22 weeks	1.1	2 years	F	14 weeks	1.4
8 months	F	14 weeks	0.9	2 years	M	20 months	2.0
9 months	F	19 weeks	0.6	2 years	F	5 weeks	1.7
9 months	F	9 weeks	1.0	2 years	M	12 weeks	1.8
9 months	M	8 weeks	1.1	3 years	F	19 weeks	1.6
10 months	F	19 weeks	1.4	3 years	M	2 weeks	1.3
12 months	M	3 weeks	1.3	3 years	F	19 weeks	1.5
12 months	F	?	1.5	4 years	F	17 weeks	1.8
13 months	M	15 weeks	1.0	4 years	F	4 weeks	1.1
14 months	F	18 weeks	1.4	6 years	F	17 weeks	1.1
14 months	M	7 weeks	0.8	7 years	M	20 weeks	1.3
14 months	F	18 weeks	1.5	7 years	F	7 years	1.4
16 months	M	21 weeks	1.4	13 years	F	6 years	1.3
16 months	F	21 weeks	1.1	13 years	F	10 years	1.4
16 months	M	19 weeks	1.2	15 years	F	24 weeks	1.2
18 months	F	22 weeks	1.6	16 years	F	13 years	1.5
18 months	M	21 weeks	1.4				
18 months	M	2 weeks	1.2	Controls			
19 months	M	5 weeks	1.1	7 years	F	....	1.0
20 months	F	10 days	1.7	8 years	F	....	1.0
20 months	F	?	2.2	9 years	F	....	0.8
20 months	M	6 days	1.6	Adult	F	....	1.5
21 months	F	?	1.6	Adult	F	....	1.2
2 years	M	9 weeks	1.1	Pool of 4 adults	..	....	1.0
2 years	M	27 weeks	1.6				

Average opsonic index of human immune poliomyelitic serum 1.34.

Ten serums were tested with No. 44; four of these were above normal and six below. Ten serums were tested with No. 707; eight were normal or above and two were below normal. No. 20 gave consistently high results. We tested the opsonizing power of 45 cases of acute and convalescent poliomyelitis on this streptococcus and found an index above normal in 39 instances. Two were normal. The other 4 cases were very young babies who showed an index above normal for that age. Thus this organism gave a high opsonic index in practically every case. The majority of these tests were made from 3-4 months after the onset of the disease.

In determining the opsonic index of normal children with Streptococcus 20, we tested the blood of 9 new-born babies from the Jewish Maternity Hospital, and of 20 inmates of the Home for Training in

Speech of Deaf Children. The children enter this school, which is located in the country, when 4 or 5 years of age and live there under close medical supervision until ready for high school or work. The very nature of their infirmity insures that they have associated but little with the children who go to the public schools and play on the street. In our opinion they furnish a group of children as free as could be found from mild and unrecognized attacks of poliomyelitis. As shown by the accompanying table we obtained a very low opsonic index with the nine new-born babies and a normal or low index in 18 of the 20 deaf children.

TABLE 2

The Opsonic Action of Normal Child Serum on Streptococcus 20

Age	Sex	Opsonic Index	Age	Sex	Opsonic Index
4 years	F	0.9	11 years	M	0.9
5 years	M	1.2	11 years	M	0.7
5 years	M	1.0	12 years	F	0.9
8 years	M	0.7	12 years	M	0.9
8 years	M	1.0	Babies:		
8 years	F	0.9	1 day	F	0.5
9 years	F	1.0	1 day	M	0.6
9 years	F	0.7	1 day	F	0.6
9 years	M	0.9	2 days	F	0.7
9 years	M	0.8	8 days	F	0.5
9 years	M	0.7	Pool of 4		
10 years	F	0.9	babies	..	0.7
10 years	M	0.7	Controls:		
10 years	M	0.9	Adult	M	1.0
11 years	F	0.9	Pool of 8		
11 years	F	1.2	adults	..	0.9

Average opsonic index of normal child serum 0.82.

DETAILS OF FURTHER INVESTIGATIONS

In order to investigate further any connection between Streptococcus 20 and poliomyelitis, we obtained blood from all the members of households in which one or more cases of poliomyelitis had occurred. Our object was to see if the patient gave a high index and also to see if any other member of the household might give a high index, suggesting a previous abortive attack of poliomyelitis. The serums were tested with the same technic used in our previous experiments, the one making the tests not knowing from which members of the household the blood was obtained.

Family 1 consisted of 6 individuals 2 of whom had had poliomyelitis. Tests were made with a serum heated to 55 C. for 10 minutes. No specific opsonin for Streptococcus 20 could be demonstrated in either patients. The serum of No. 5, a 16-year-old girl, contained a

considerable amount of thermostable opsonin. She never had had recognized poliomyelitis.

In Family 2, the patient gave an index of 0.9, and a girl 11 years old, with no history of poliomyelitis, gave an index of 1.5.

TABLE 3

The Opsonic Action on Streptococcus 20 of Serum of Members of Households in which a Case of Acute Anterior Poliomyelitis Had Occurred

	No.	Age Years	Sex	History	Unheated Serum		Serum Heated 55 C. 10 Min.; Phagocyte Count
					Phagocyte Count	Opsonic Index	
Family 1	1	12	M	Normal......................	...	...	0.8
	2	4	M	A. A. P., 17 weeks*...........	...	...	1.0
	3	7	M	A. A. P., 18 weeks............	...	...	1.3
	4	14	F	Normal......................	...	...	0.8
	5	18	F	Normal......................	...	...	0.8
	6	16	F	Normal......................	...	...	5.0
Family 2	1	9	M	A. A. P.	6.5	0.9	0.6
	2	11	F	Normal......................	11.0	1.6	1.0
	3	45	F	Normal......................	4.0	0.6	0.3
	4	33	F	Normal......................	6.0	0.9	0.6
	5	4	M	Normal......................	8.0	1.1	0.6
	6	2	M	Normal......................	2.0	0.3	0.6
	Adult		M	Normal, control..............	7.2	1.0	
Family 3	1	3	F	A. A. P., 30 weeks............	7.0	0.9	
	2	20	F	Normal......................	6.0	0.7	
	3	27	M	Normal...	17.0	2.1	
	4	22	F	Normal......................	7.0	0.9	
	5	62	M	Normal......................	5.0	0.6	
	6	27	M	Normal......................	9.0	1.1	
	Adult		M	Normal, control..............	8.0	1.0	
Family 4	1	26	F	Normal......................	13.0	1.5	
	2	4	M	Normal......................	10.0	1.2	
	3	2	F	A. A. P.	9.0	1.1	
	4	3	M	Normal......................	11.0	1.3	
	5	5	M	Normal......................	13.0	1.5	
	6	30	M	Normal......................	8.5	1.0	
	Adult		M	Normal, control..............	8.5	1.0	
Family 5	1	12	M	Cerebrospinal meningitis, 30 days......................	1.0	0.1	
	2	14	F	Cerebrospinal meningitis, 9 days......................	3.0	0.4	
	3	11	M	Normal......................	1.0	0.1	
	4	9	F	Normal......................	1.5	0.2	
	5	6	M	Normal......................	6.0	0.9	
	6	Adult	F	Normal......................	1.0	0.1	
	7	2	M	A. A. P., 28 days..............	2.0	0.3	
	Adult		M	Normal, control..............	7.0	1.0	

* Acute anterior poliomyelitis, 17 weeks since onset.

In Family 3, the patient gave an index of 0.9. One member of the household, an employee, gave an unusually high index, 2.1. Here again there was no history of the man having had poliomyelitis.

Five of the 6 members of Family 4 gave indexes above normal, the patient's being 1.1.

Family 5, with which are included 2 acute cases of cerebrospinal meningitis for purposes of comparison, gave very low indexes. Patient 7 was in the acute stage of poliomyelitis. The fact that this household, in which the case was an acute one, gave unusually low indexes is interesting.

We did not find the serums of the patients characterized by the presence of a large amount of opsonin. In two of the households, individuals with no history of poliomyelitis gave quite high indexes. We are unable at present to offer any explanation of these findings, having made no investigation as to how soon after onset the index begins to rise and as to how long it remains high.

In Families 1 and 3, the patients had been tested some months before, and at that time had given indexes above normal. They are included in the series of tests made with Streptococcus 20 recorded above. When examined the second time, much of the increased opsonin had disappeared. Such a gradual loss of opsonic power has been demonstrated by Mathers and Tunnicliff,[6] who used a different technic and an organism which we have shown elsewhere to be identical with Streptococcus 20.

The fact that the serum of a large percentage of patients with poliomyelitis give a high opsonic index with streptococci isolated from poliomyelitic material may not have any direct bearing on the etiology of that disease. But what it does clearly suggest, if not prove, is that these streptococci are not preagonal or post-mortem invaders. At some time before, during or after the acute febrile stage of poliomyelitis these streptococci must have been in intimate and sufficiently prolonged contact with the tissues of the patient to stimulate the production of antisubstances. Further evidence of this possibility is shown by positive complement fixation reactions observed by Kolmer and Freese[7] with the serum of a small percentage of persons in the late stages of acute anterior poliomyelitis and polyvalent antigens of streptococci.

Serum from convalescent poliomyelitic patients does not give a high opsonic index with all streptococci. The reaction, so far as we have studied, is confined to strains of rather large organisms occurring in short chains isolated from poliomyelitic material. Mathers and Tunnicliff[6] have likewise shown that the rise and fall of the opsonic power against the poliomyelitic strain is not paralleled by a similar

[6] Jour. Am. Med. Assn., 1916, 67, p. 1935.
[7] Jour. Immunol., 1916, 2, p. 327.

curve when the same serum is tested with the Streptococcus pyogenes. We have found the same to be true not only with streptococci from other sources but with staphylococci, diphtheroids and bacilli of the colon group found in poliomyelitic material. These latter would seem to have established themselves post mortem.

SUMMARY

In addition to and distinct from the globoid bodies, there is frequently present in poliomyelitic material a rather large streptococcus, occurring in short chains.

The serums of a large percentage of patients with poliomyelitis give high opsonic indexes with this streptococcus, but not with streptococci from nonpoliomyelitic sources, nor with staphylococci, diphtheroids and gram-negative bacilli obtained from poliomyelitic material.

STUDIES IN EPIDEMIC POLIOMYELITIS

III. COMPARATIVE STUDIES OF COCCI ISOLATED FROM POLIOMYELITIS

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Cocci have been isolated repeatedly from poliomyelitic material, many observers in various localities during the past year having described strains they have found. A comparative study of these strains has seemed desirable to determine whether it is one organism or several organisms the different workers have found, and, if several, what points the different strains have in common.

Accordingly we assembled eight strains and studied their behavior when examined by elementary bacteriologic methods, and also by methods aimed to establish their more intimate functional relationships.

Rosenow, Towne and Wheeler[1] supplied us with 2 strains: No. 44, "the fourth subculture of a strain from a monkey having poliomyelitis following injection of a filtrate of a virus of a monkey that was paralyzed with a filtrate of a virus," and No. 707, "a strain isolated from a single colony from the spinal cord of a case of poliomyelitis in man . . . now in the ninth subculture."

Mathers[2] furnished us with two: Nos. 1 and 7, both from human poliomyelitic material.

Nuzum and Herzog[3] sent us two: No. 1, "from the spinal fluid of a monkey," and No. 2, "from human spinal fluid."

From Dixon and Rucker[4] of Philadelphia we obtained one: No. M 61, from human poliomyelitic material.

From Kolmer, Brown and Freese[5] we obtained one : No. 20, isolated from the pons of a patient who died of poliomyelitis on the seventh day.

Cocci that we have proved to be identical with Streptococcus 20 we have ourselves isolated in the course of cultural work. Glycerinated poliomyelitic nervous tissue from two human cases was emulsified, and one emulsion passed through a Chamberland and the other through a Reichel filter. Cultures from both filtrates gave No. 20. We have, in association with Kolmer, described elsewhere the finding of the globoid bodies of Flexner in these two lots of poliomyelitic material. In another instance, portions of the brain and cord of a

[1] Jour. Am. Med. Assn., 1916, 67, p. 1202.
[2] Jour. Infect. Dis., 1917, 20, p. 113.
[3] Jour. Am. Med. Assn., 1916, 69, p. 1205.
[4] Report of Commissioner of Health, State of Penna., 1907, p. 434.
[5] Jour. Exper. Med., 1917, 25, p. 789.

monkey with typical anterior poliomyelitis following intracerebral injection of a human virus were distributed among 20 tubes of kidney-acetic fluid medium overlaid with oil. In 9 tubes we found pure cultures of the coccus identical with Streptococcus 20. From the brain of this monkey we were able also to isolate Flexner's globoid bodies.

All 8 strains grow well in air. On glucose-agar slants made from infusion broth, Streptococcus 20, Nuzum's No. 1, and No. M 61 grew abundantly, forming a heavy, smooth, white growth. The other 5 strains gave the fine, discrete, yellowish white growth which is usually associated with the pathogenic streptococci.

When stained smears from broth cultures are examined no constant differences can be detected among the 8 strains. They are all gram-positive and appear singly, as diplococci, in short chains and in small groups. They are of much the same size and no distinct capsule can be made out. All the strains are nonmotile.

When broth cultures are eamined in a hanging drop the 8 strains, according to Chester's[6] classification, fall into 2 groups. No. M 61 and Nuzum's No. 1 show division in more than one direction of space, the cocci appear singly, in pairs, in threes, and in small clusters. The other 6 strains show division in one direction of space only and appear singly, in pairs, and in short chains, but never in threes or in groups, and would be classed with the streptococci. Further investigations were confined to the latter group which showed division in 1 direction of space only, No. M 61 and Nuzum's No. 1 being eliminated.

To determine what action these streptococci have on red blood corpuscles the method described by Lyall[7] was followed. One c.c. of a 2½% suspension of washed sheep corpuscles and 2 c.c. of normal salt solution were placed in test tubes and 0.5 c.c. of 24-hour broth cultures of the streptococci added. The tubes were shaken, placed in a water-bath at 27 C. for 1 hour, then placed in the refrigerator over night. By this method hemolysis of the sheep cells, the production of methemoglobin, or the absence of any action of the bacteria on the corpuscles can be observed.

Strains 1 (Mather's), 7, and 44 were actively hemolytic; Strain 2 gave the brown color of methemoglobin; Strains 20 and 707 had no action on the blood cells.

The action of the 6 strains on inulin, raffinose and salicin was tested. The medium used was sheep-serum-water containing litmus as an indicator, to which was added 1% of the carbohydrate tested. Twenty-four-hour broth cultures of each of the 6 strains were planted in the tubes with a pipet. The tubes were observed for 5 days. Inulin was not attacked by any strain. No. 44 fermented both raffinose and salicin. Nos. 707, 2, and 20 fermented salicin only, while Mather's strains (Nos. 1 and 7) did not produce acid with any one of the 3 carbohydrates.

The fact that none of these strains fermented inulin in our hands, that they are all short chain streptococci, and that none of them produce hydrogen sulphide, would serve to exclude them from any of the Andrewes and Horder's[8] types except Streptococcus mitis or Strep-

[6] A Manual of Determinative Bacteriology, 1914.

[7] Jour. Med. Research, 1914, 30, p. 487.

[8] Systematic Relationships of the Coccaceae, 1908.

tococcus salivarius, the common habitat of both of which is the human mouth.

As Wright[9] has pointed out, in the serum of animals that have been artificially immunized against a given organism there exist opsonins which resist the heating of the serum to 55 or 60 C. for 10 minutes. We accordingly determined to see if an investigation of the poliomyelitic streptococci along this line would bring out any information of value. To this end we immunized 3 rabbits, each with one of 3 strains of the poliomyelitic streptococci, and tested the opsonizing power of the heated serum of each rabbit against the other strains.

In performing the opsonic test we followed strictly the technic of Wright.[10] The serums were tested within 24 hours after being drawn and were heated in a water-bath at 50 C. for 10 minutes. This amount of heating of normal serum leaves just sufficient opsonin to cause an average intake of less than one coccus per leukocyte. A suspension of cocci containing from 2-4 billion

TABLE 1

OPSONIC ACTION OF ANTIPNEUMOCOCCIC HORSE SERUM ON TYPES 1, 2, AND 3 OF PNEUMOCOCCI

Serum Heated	Average per Leukocyte of Type 1 Pneumococci	Average per Leukocyte of Type 2 Pneumococci	Average per Leukocyte of Type 3 Pneumococci
Type 1 Serum	5.0	0.0	0.5
Type 2 Serum	0.0	5.0	0.3

per c.c. is of a satisfactory concentration. When a heavier suspension of organisms is used it is necessary to heat to a higher degree to achieve the desired result of less than one coccus per leukocyte. We have found that a careful adjustment must be made between the amount of heating and the number of organisms in the suspension in order to secure clear-cut results. As a preliminary to each set of tests it is necessary to make a "trial-trip," using heated normal serum, and see that just sufficient phagocytosis remains to be assured that any increased opsonin in the serum will not be influenced by the heating.

In order to see with what delicacy we might expect distinctions to be drawn by this method, we made some preliminary experiments with Types 1, 2 and 3 of pneumococci, and the serum of horses that had been highly immunized against Types 1 and 2. The laboratory of the Bureau of Health of Philadelphia furnished us with the 3 types of pneumococci and with fresh serum. Each serum was tested against

[9] Studies on Immunization, 1909.
[10] Technique of the Teat and Capillary Glass Tube, 1912.

the 3 types and increased opsonin was found present only in the serum for the type with which the animal had been injected.

In view of this result, we felt that if the serum of an animal that had been immunized with one poliomyelitic streptococcus contained increased opsonin for another strain, the 2 strains might rightly be considered as more closely related to each other than are the 3 types of pneumococci. Even should no phagocytosis follow such cross-testing we would still not be justified in denying a close relationship. In the absence of any classification of streptococci to which we can refer for final decision, our results must be stated in relative terms, namely, that 2 strains are more closely related than are the different types of pneumococci or that they are less closely related.

Three rabbits were injected intravenously with living cultures, one with No. 20, one with No. 44, and one with No. 707. Three large injections were given, and, after allowing time for recovery from the negative phase, the serum of each rabbit was tested against the strain with which it had been immunized. With unheated serum such numbers of cocci were taken up that often a count was impossible. When serum heated to 55 C. for 10 minutes was employed, sufficient thermostabile opsonin remained to allow an active phagocytosis.

TABLE 2

Opsonic Action of Unheated and Heated Serum of Immunized Rabbits on the Strain of Poliomyelitic Streptococci with which They Were Injected

	Serum 20 Plus Streptococcus 20	Serum 44 Plus Streptococcus 44	Serum 707 Plus Streptococcus 707
Unheated.....................	Uncountable *	17	46
Heated 55 C. for 10 Minutes	16	5	15

* Average per leukocyte.

Assured that each of the serums contained thermostabile opsonin for the strain with which the rabbit had been injected, we proceeded to test each serum against suspensions of the 6 strains of streptococci. In each case the suspension was controlled by using normal rabbit serum heated to 55 C. for 10 minutes.

Examining the results of the tests in which the serum of the rabbit immunized with Streptococcus 20 was used, we see that Strains 20, 1, 7, and 2 were all phagocytized readily, after having been acted on by this immune serum. Evidently these 4 strains are the same so far as this method of investigation is concerned. With Strains 44 and 707 phagocytosis did not occur.

When the serum of the rabbit immunized with Strain 44 was used, phagocytosis took place with Strains 44 and with 707, but not with any of the other strains. It was observed in working with Strain 44 that when the cocci had been acted on by serum they very often became greatly swollen and stained very deeply. This did not occur with any culture except No. 44.

Serum from the rabbit immunized with Strain 707 causes phagocytosis of Strains 707 and 2, but not of any other strain.

We may conclude from this series of tests that Strains 20, 1, 7, and 2 are identical or are more closely related than are the 3 types of pneumococci. The results here are clear-cut and unambiguous. With Strain 707 we are left in some doubt as to the relation it bears to Strains 20, 1, 7, and 2. Possibly it should be grouped with them.

TABLE 3

Opsonic Action of Immune Rabbit Serum on Six Strains of Poliomyelitic Streptococci from Various Localities

Suspension of Streptococcus	Serum Heated to 55 C. for 10 Minutes			
	Serum of Rabbit Immunized with No. 20	Serum of Rabbit Immunized with No. 44	Serum of Rabbit Immunized with No. 707	Serum of Normal Rabbit
20	16.0 *	0.7	0.7	0.3
44	0.5	5.0	0.7	0.9
707	0.7	2.0	15.0	0.6
1	3.0	0.6	0.9	0.3
7	9.0	0.1	0.7	0.6
2	10.0	0.0	13.0	0.3

* Average per leukocyte.

Strain 44 would seem by this method of classification to be placed in a separate group from Mather's, Nuzum's and Kolmer's strains.

Agglutination with the coccaceae is admittedly unsatisfactory. The production of agglutinins by the injection of cocci is slow and the agglutinative titer is not high. However, in the endeavor to make our examination of the poliomyelitic cocci as complete as possible, we made a series of agglutination tests with immune rabbit serum, testing each serum with each of the 6 strains of streptococci. The mixtures of serum, normal salt solution and cocci were made and incubated in capillary glass tubes according to the method described by Wright.[10] The results were read after the mixtures had been incubated 24 hours.

The results of the agglutinations parallel closely the results of the opsonic tests, but the distinctions drawn are less fine. The agglutination method detects no difference whatever between Strains 20, 1, 7, 2, and 707. By the opsonic method, it will be remembered, the identity of Strain 707 with the other 4 was uncertain. In the agglutinations, Strain 44 again stands out as a strain of another type.

TABLE 4

Agglutination of Six Strains of Poliomyelitic Streptococci by the Serums of Rabbits Immunized with Different Strains

	Dilutions				
	1:4	1:8	1:16	1:32	Control
Serum of Rabbit Immunized with Strain 20 plus					
Suspension of No. 20	+	+	+	+	—
Suspension of No. 44	—	—	—	—	—
Suspension of No. 707	+	+	+	+	—
Suspension of No. 1	+	+	+	+	—
Suspension of No. 7	+	+	+	—	—
Suspension of No. 2	+	+	+	+	—
Serum of Rabbit Immunized with Strain 44 plus					
Suspension of No. 20	+	±	—	—	—
Suspension of No. 44	+	—	—	—	—
Suspension of No. 707	+	—	—	—	—
Suspension of No. 1	—	—	—	—	—
Suspension of No. 7	—	—	—	—	—
Suspension of No. 2	±	—	—	—	—
Serum of Rabbit Immunized with Strain 707 plus					
Suspension of No. 20	+	+	+	—	—
Suspension of No. 44	—	—	—	—	—
Suspension of No. 707	+	+	—	—	—
Suspension of No. 1	+	—	+	—	—
Suspension of No. 7	+	—	—	—	—
Suspension of No. 2	+	+	+	+	—
Serum of Normal Rabbit plus					
Suspension of No. 20	±	—	—	—	—
Suspension of No. 44	—	—	—	—	—
Suspension of No. 707	±	—	—	—	—
Suspension of No. 1	—	—	—	—	—
Suspension of No. 7	—	—	—	—	—
Suspension of No. 2	—	—	—	—	—

SUMMARY

With the object of determining whether it is one strain of streptococci so commonly found associated with poliomyelitis, or several, we secured cultures from investigators who have isolated strains from poliomyelitis material in various parts of the country. We have grown them side by side on the same mediums. We have applied to them fermentative and other cultural tests found useful in the classification of streptococci. We have immunized rabbits with strains from differ-

ent sources and with the immune serum so obtained have made opsonic tests on each strain to see if the heated serum giving phagocytosis with the strain with which the rabbit was injected will also cause phagocytosis of the other strains. With these same immune rabbit serums we also made cross agglutination tests.

The six strains of streptococci examined by cultural methods appear to be identical or closely related.

When examined by methods tending to bring out their functional activities Kolmer's strain, Mather's strains, and Nuzum's strain from human spinal fluid are identical. Rosenow's Strain 44 exhibits differences from the first group which are at least as great as the differences between Types 1 and 2 of pneumococci. Rosenow's Strain 707 gives limited reactions with some members of both of the 2 former groups.

While under strict anaerobic conditions the prolonged cultivation of streptococci results in a diminution of size and a tendency to become gram-negative, yet the biologic and morphologic differences between the globoid bodies and the small form of streptococci appear to be distinct.

OBSERVATIONS ON THE INCIDENCE OF INTESTINAL SPIROCHETES IN THE PHILIPPINE ISLANDS

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Nearly two years ago our attention was attracted by the presence of spirochetes in the feces of several patients under examination, and it was decided to make a systematic search for them in a series of cases of intestinal endamebiasis. One of the ideas in our minds at the time was that spirochetes in association with endamebae might cause lesions which respond to treatment with salvarsan. It is recognized that some cases of intestinal endamebiasis which are refractory to other forms of treatment do respond to the treatment with salvarsan, and it was thought that this might furnish an explanation.

Spirochetes have been found in the feces of dysenteric and nondysenteric cases by several observers since 1880. Among these observers are Kowalski,[1] Escherich,[2] Lustig and Giaxa,[3] Gruber,[3] Babes,[3] Fürbringer,[4] Kirchner,[3] Abel,[5] Aufrecht,[6] Rechtsamer,[7] Günther,[8] Le Dantec,[9] Löwenthal,[10] Mühlens,[11] Rosanow,[12] Werner,[13] Courmont and Lesieur,[14] Leber and v. Prowazek,[15] Camermeyer,[16] J. G. and D. Thomson,[17] Fantham and Porter,[18] Fantham,[19] Macfie,[20] and Carter.[21]

Since the beginning of our work several reports on the occurrence of intestinal spirochetes have appeared, prominent among these being the works of Fantham, Macfie, and Carter.

[1] Centralbl. f. Bakteriol., 1, 1894, 16, p. 321. Wien. klin. Wchnschr., 1893, 6, p. 888.
[2] Münch. ärztl. Intell.-Blatt., 1884, 51. Centralbl. f. Bakteriol., 1, 1894, 15, p. 408.
[3] Wien. med. Wchnschr., 1886, 36, pp. 342, 383, 423. Centralbl. f. Bakteriol., 1, 1894, 15, p. 721.
[4] Deutsch. med. Wchnschr., 1892, 18, p. 768.
[5] Centralbl. f. Bakteriol., 1, 1894, 15, p. 213.
[6] Ibid., p. 405.
[7] Ibid., p. 795.
[8] Einführung in das Studium der Bakteriologie, 1898.
[9] Comp. rend. Soc. de biol., 1903, 55, p. 617.
[10] Berl. klin. Wchnschr., 1906, 43, p. 283.
[11] Ztschr. f. Hyg. u. Infectionskrankh., 1907, 57, p. 405.
[12] Russk. Vrach., 1909, No. 10.
[13] Centralbl. f. Bakteriol., I, O., 1909, 52, p. 241.
[14] Lyon Méd., 1911, p. 19.
[15] Arch. f. Schiffs-u. Tropen-Hyg., 1911, 13, p. 421.
[16] Arch. f. Schiffs-u. Tropen-Hyg., 1912, 16, p. 84.
[17] Proc. Roy. Soc. Med., 1914, 7, p. 45.
[18] Proc. Cambridge Phil. Soc., 1915, 18, p. 184.
[19] Brit. Med. Jour., 1916, 1, p. 815.
[20] Ann. Trop. Med. and Parasit., 1917, 10, p. 391.
[21] Lancet, 1917, 192, p. 336.

Fantham found them with great frequence, and concerning their identity says: "It is probable that the earlier authors . . . were dealing with the same spirochaete, which exhibited morphological variation due to the processes of growth and division. The first name proposed by Werner, namely, *S. eurygyrata,* will then be valid. It is therefore necessary to amend the definition of the organism as follows: *Spirochaeta eurygyrata* has tapering ends, measures up to 15 microns long and is about 0.25 micron broad. It contains a diffuse nucleus consisting of chromatinic granules. The number of coils or waves is variable, depending on the rate of movement and thickness of the organism.

"*Spirochaeta eurygyrata,* Werner, emend. Fantham, may occur in the stools of dysenteric and apparently healthy persons. The organism, which has pointed ends, measures from 3 microns to 15 microns in length by about 0.25 micron in breadth.

"The so-called spirilla, mentioned by some of the earlier workers as occurring occasionally in cholera motions, are included under the name *Spirochaeta eurygyrata.*

"The number of coils in a spirochaeta is not a specific character but is variable, and is primarily an index of its rate of motion, being also partly dependent on the thickness of the organism."

Macfie studied this question in Africa and came to the following conclusions:

"*Spirochaeta eurygyrata* Werner, emend. Fantham, is well nigh an invariable inhabitant of the human intestine at Accra, on the Gold Coast, West Africa.

"Similar, if not identical, organisms are found also in the intestines of various animals.

"As a rule, the spirochaetes appear to be nonpathogenic—that is to say, their presence is not incompatible with a normal state of health, but under certain conditions they multiply so enormously that it is difficult to believe that they can be entirely benign."

Carter in the Liverpool School of Tropical Medicine studied smears from the stools of 554 patients admitted into hospital for dysentery or related diseases, and of these 313, or 56.5%, showed spirochetes. Some of his cases were examined more than once and "latent positives" were thus found. Computing from the number thus found he thinks that the number of "positives" should be raised to 346, or 62.4%. He also examined for spirochetes 100 nondysenteric patients, suffering from nonintestinal diseases, and 41% were shown to be infected. Here he thinks an appreciable number of "latent positives" were included among the "negatives." In view of his results he concludes that spirochetal infections of the alimentary canal are almost, if not quite, as prevalent in nondysenteric as in dysenteric patients. He found spirochetal infections frequently mixed with single or multiple protozoal infections of the alimentary tract, but concludes that there would appear to be no correlation between the presence of these organisms and the occurrence of any of the commoner protozoa in the stools.

Wolbach[22] has reported a spirochete from human feces which he recovered in pure culture after passage through a Berkefeld filter. This spirochete he was able to cultivate in slightly acid meat-infusion-broth. He has also cultivated it on agar-agar. We are unable to determine at the present time if the organism seen by us in Manila is identical with that described by Wolbach.

Many of the earlier workers found the spirochetes in association with the cholera vibrio and some suggested that the spirochetes found an especially good medium for growth in the cholera intestine. In the light of the more recent

22 Amer. Jour. Trop. Dis. and Prev. Med., 1915, 2, p. 494.

work on the subject, it appears to us that the fact that so many of the earlier workers found intestinal spirochetes in cholera cases is accounted for by the fact that it has been during cholera epidemics that the largest number of examinations of feces have been made. Among the places from which these intestinal spirochetes have been found are England, Trieste, Bordeaux, Belgian Congo, German S. W. Africa, Gold Coast, Berlin, Hamburg, Magdeburg, Greifswald, Gallipoli, Flanders, Caucasus, Tonkin, Samoa, and to this list we add the Philippine Islands.

The results of our own work on this subject are reported in order to add to the knowledge of the geographic distribution of intestinal spirochetosis, and to give what data we now have, in case this condition should later assume a significance which is not attributed to it at the present time.

For the purpose of making a study of this spirochete, a series of 50 stools was obtained from the laboratory of clinical pathology at the Philippine General Hospital, the intention mainly being to determine the incidence of the infection and develop a line of attack on the problem in general.

In many cases the specimens were not received at the laboratory until many hours had elapsed after they had been passed. These specimens, as might be expected, did not offer the best material on which to make a differential diagnosis between the various endamebae. Therefore, no attempt was made to diagnose species and the various endamebae found were simply recorded under the generic name. No difficulty was experienced in determining the identity of trichomonas or the various eggs of helminths which were found from time to time.

The material was first examined fresh, and in the case of formed stools a little physiologic sodium chlorid solution was used to emulsify the feces. Two permanent preparations were made from each stool and all smears were fixed wet, either in Bouin's picro-aceto-formol solution, or sublimate-acetic fluid. Staining was done by the Heidenhain iron-hematoxylin method or with Delafield's hematoxylin, or the modified Delafield solution made according to the formula of one of us.[23] All three stains gave a clear demonstration of the spirochete, and, indeed, this spirochete seems particularly amenable to treatment by the standard cytological methods. The best demonstration of the chromatinic granules was obtained by treatment with the modified Delafield solution.

In the larger proportion of cases, that is to say, the forms measuring 4 microns or more, there was little difficulty in observing the living

[23] Shumway: Jour. Exper. Zool., 1917, 22, 532.

spirochetes in the fresh preparations, by direct illumination.   In a number of cases, however, the preparation was swarming with bacilli 4-6 microns in length, and some care was necessary to distinguish them

Length in Microns
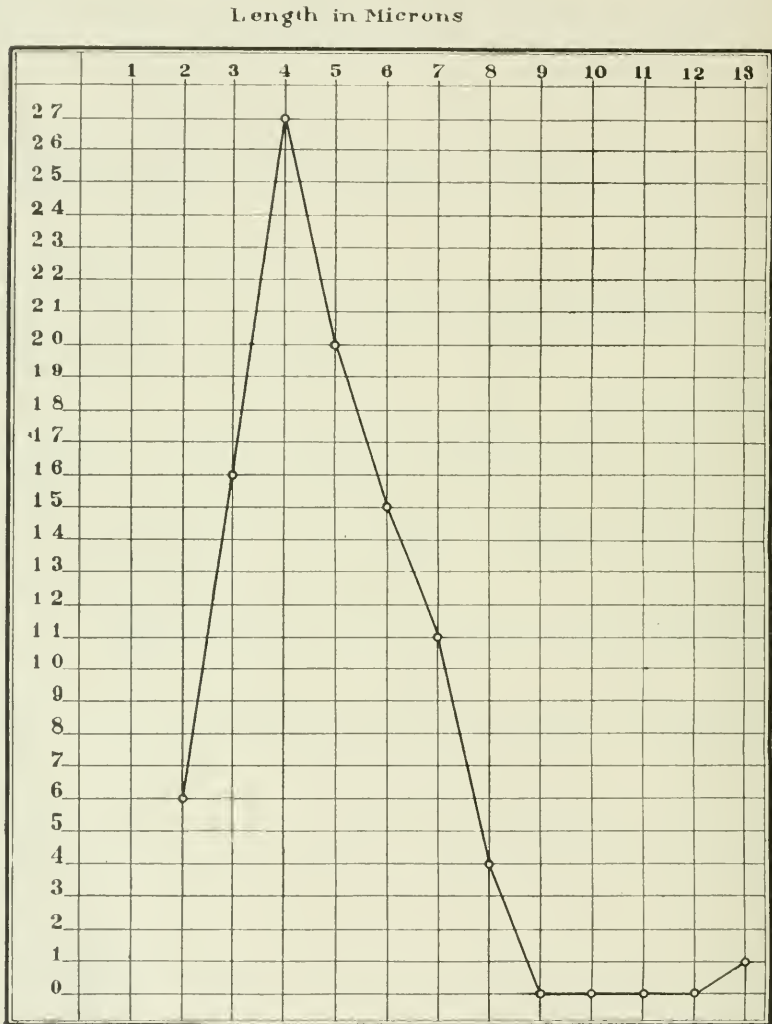
Chart 1.   Measurements of 100 intestinal spirochetes.

from spirochetes.   Sometimes the appearances in the stained preparations gave rise to the same doubt, especially if the bacillus had become bent or twisted in making the film.   Such forms were carefully

excluded and a positive diagnosis of spirochetes was made only on the presence of forms which were unmistakably spirochetes.

Measurements were made of 100 individuals selected at random from various stained preparations. The method of Stephens was used, and the measurements were made under a magnification of about 1,400 diameters. The results have been plotted and are shown on Chart I. It will be seen that the range in size is from 2 microns up to 8 microns, but that a single individual measured 13 microns. This latter bore every appearance of being a dividing individual; it was partly incurved and showed a distinct thinning at the middle, and suggests an explanation for the presence of a flagellum-like body seen at one extremity of two individuals.

Fifty stools were examined in the series, representing 46 separate hospital cases. Of these cases, thirty-five (73%) were positive for spirochetes. There were seventeen cases (48%) which showed a concomitant infection with endameba, and scattered infections with Trichomonas intestinalis and the more common helminths.

The patients from whom these stools were obtained were undergoing treatment for various ailments. Of the 35 which were positive for spirochetes, 10 patients were receiving treatment for endamebic dysentery.

Unfortunately, in the greater proportion of cases, it was not possible to examine more than one stool of a given patient. In 4 cases, however, two stools were received. In 2 of these cases, one examination was positive and the other negative. In the other 2 cases, each examination yielded positive results.

THE NECESSITY OF CARBON DIOXID FOR THE GROWTH OF B. TUBERCULOSIS

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Last year one of us noted that if the respiratory CO_2, evolved by a freshly planted virulent culture of the tubercle bacillus, was removed, growth did not take place. The bacilli remained in a nonreproductive state but viable, as shown by guinea-pig inoculation two weeks later.

The culture was isolated, in February 1917, by Dr. John Hermanies, from postmortem pulmonary lesions in man, by guinea-pig inoculation and cultivation on rabbit blood agar. Repeated tests made shortly after isolation and off and on up to the present time have confirmed the original observation regarding the necessity of CO_2. In the earlier tests the respiratory CO_2 was absorbed by joining the culture tube to another test tube, containing from 5-10 c c of saturated barium hydroxid solution, by means of rubber tubing. As controls, similar cultures were attached to tubes of distilled water. As the pellicle of barium hydroxid formed, it was shaken down daily. When this shaking down of the pellicle was performed faithfully, the growth was completely inhibited. But when the pellicle was allowed to stand, as it was usually in the later stages of the observations, the absorption was interfered with, CO_2 accumulated, and growth then usually began as isolated islands. This differed markedly from the growth in the water tubes which started all over the inoculated surface and soon yielded a confluent nodular layer. In our later experiments we have used sodium hydroxid as this requires no attention. Figure 1 shows the results of growth against sodium hydroxid and water, respectively.

Most of these experiments have been performed on nutrient agar made up from the extract of lean beef with 1% Witte's peptone, 0.5% Na_2HPO_4 and 6% glycerin. This was neutralized with normal sodium hydroxid to a reaction of $\mp 1.5 - \mp 2.0$ phenolphthalein. It was tubed in 5 c c quantities and autoclaved. To each tube was then added 0.5 c c defibrinated rabbit blood. On this medium, in the tubes attached to water, the growth was luxuriant and confluent in 2 to 3 weeks at

37 C. After inoculation there was always, however, a period of "lag" during which no proliferation could be noted.

We are now engaged in a closer analysis of optimum CO_2 pressure which will also be supplemented by a study of the oxygen requirements. Reference to the accompanying table will show that one might be led
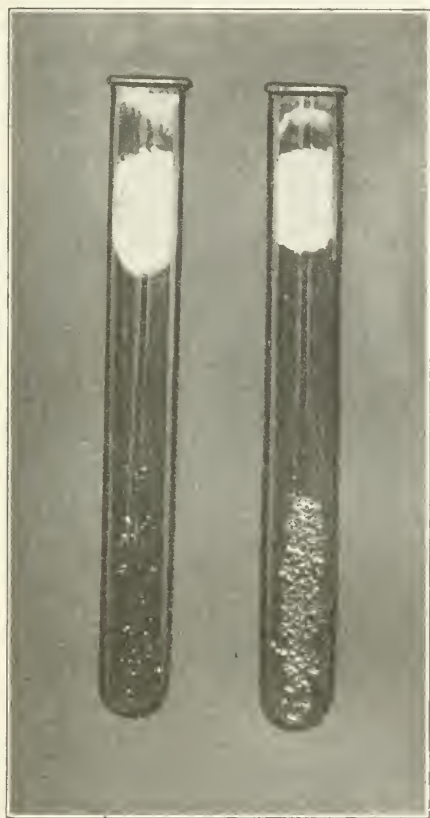
Fig. 1.—Showing the effect of attaching cultures to sodium hydroxid solution and water, respectively.

to the belief that "lag" is a latent period during which CO_2 accumulates to an optimum concentration. Accompanying this increase in CO_2 pressure is a gradually diminished O pressure. When both O and CO_2 pressures reach an optimal point, growth starts. For example, as shown in the table, Tube III under an initial pressure of 17 mm. of CO_2 on December 31, showed approximately the same growth as Tube

VI did on December 26, which was under an initial pressure of 47 mm. It should be noted that the growths in this series all occurred with certain limits of CO_2 pressure; e. g., Tubes III-VI. In Tubes IV and V, it will be noted that the CO_2 escaped and, while growth commenced, it soon ceased. In Tube I, which was under atmospheric (laboratory, 7 mm.) CO_2 pressure and in Tube VII in which the CO_2 pressure was 77 mm., there was no growth. We have realized the necessity of investigating the need for oxygen. All the recorded observations we have seen emphasize the necessity of a supply of free oxygen for the growth of B. tuberculosis on artificial mediums. Just

Comparative Tests which Illustrate the Effect of Different CO_2 Pressures on the Growth of B. Tuberculosis

Dates of Observation	December 18	December 26		December 31		January 3		January 8		
Barometer = 750 mm. Temperature = 20 C.	CO_2 in mm.	Sapro-phytic	Non-sapro-phytic	Sapro-phytic	Non-sapro-phytic	Sapro-phytic	Non-sapro-phytic	Sapro-phytic	Non-sapro-phytic	CO_2 %
I	H_2O 7.7△	+	0	+	0	++	0	+++	+	6.3
II	NaOH 0	+	0	+	0	+	0	+	0	0.0
III	17	++	+	+++	+	+++ +++	++	+++ +++	++	8.25
IV	27	++	++	++	++	++	++	+++	++	2.0*
V	37	+	++	++	++	++	++	++	++	1.2*
VI	47	+++	+	+++	+	+ +++	++	++ +++	++	6.8
VII	77	0	0	0	0	0	0	0	0	13.0

△ Room pressure = 7.7 mm.
+ sign indicates the degree of growth.
+++ means a luxuriant confluent nodular layer of growth.
* evidently CO_2 escaped.

how much is needed has not been determined apparently. However, the experiments of Adams[1] show that concentrations above the atmospheric inhibit growth on artificial mediums. Now, in a closed system such as has been described above, not only does CO_2 accumulate but the oxygen supply rapidly diminishes. However, there is usually enough oxygen present to yield a confluent layer of growth.

In the accompanying table, we refer to saprophytic and nonsaprophytic strains. The nonsaprophytic is the one with which most of our experiments were performed. The saprophytic is called such because it grows a little more luxuriantly on all mediums than the parent strain

[1] Biochem. Jour., 1912, 6, p. 297.

from which it was derived. It originated in the following interesting manner:

A freshly inoculated transplant, on the glycerin rabbit's blood agar previously described, was attached to a tube of barium hydroxid, Oct. 13, 1917, and kept in the incubator at 35-37 C. until Dec. 10, 1917. It had not been disturbed for at least a month. The hydroxid tube was covered with a thick scum of the carbonate and the culture showed 9 islands of growth from 2-4 mm. in diameter. Four of the layer islands, which were of a greenish color, showed dryish wrinkled salmon-colored outgrowths from the edges—thus producing areas of growth from 10-12 mm. in diameter. This outgrowth was composed of completely acid-fast rods resembling the tubercle bacillus and not differing in morphology markedly from the bacilli in the central raised portions of the islands. Thinking that this might be a variant it was subcultured to mediums of the same composition but neutralized with sodium carbonate. In 6 days it yielded a growth as luxuriant as the original culture from which it was derived would yield in 3-4 weeks. Transplants from the central portion of the above areas grew extremely slowly on this medium. This saprophytic variant has shown another peculiarity in that its grows luxuriantly at partial tension, namely, when joined to a culture of B. subtilis, whereas experiments like this performed on several occasions previously with the parent strain always yielded negative results.

OBSERVATIONS ON THE BACTERIOLOGY
OF CHOREA

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The frequent association or sequence of tonsillitis, acute rheumatic fever, endocarditis, and chorea in children is an ancient observation in clinical medicine. The etiology of chorea has been explained on several theories, one of which is that it is an infection.

Many investigators have obtained bacteria in blood cultures in chorea before and after death, but in a large number of these cases, rheumatic fever or acute cardiac involvement was associated. There is very little written of the bacteriology of uncomplicated chorea.

Pianese[1] isolated a bacillus from a postmortem blood culture. Dana demonstrated microscopically a diplococcus in the meninges of a patient dead of chorea, and Westphall, Wassermann, and Malkoff[3] isolated diplococci from the brain in chorea.

Poynton and Payne[4] examined 4 fatal cases of rheumatism, 3 of which had chorea at the time of death. Diplococci were cultivated from the pia mater in 3 cases, and from the brain in 1.

Donath[5] isolated Staphylococcus albus from the blood in chorea after death.

In 2 patients, 1 of whom had endocarditis and decompensation, Richards[6] isolated a coccus which grew in short chains, and produced green pin-point colonies on blood agar in 6-8 days.

Apert[7] and Richter[8] each isolated diplococcus from the blood of a patient who had chorea during life.

Camisa[9] reports 9 cases, from 6 of which cultures of the blood gave cocci arranged in short chains and pairs, all having the same morphologic and cultural characteristics. All these cases, however, showed evidence of cardiac involvement.

Donath[5] cultured the blood of 7 severe cases, and isolated Staphylococcus albus in 4, and Staphylococcus aureus in 1.

Morse and Floyd[10] examined 31 blood cultures taken from 26 patients, and found a diphtheroid bacillus in 1, cocci growing in short chains in 2, and a green

[1] La Riforma Med., 1891.
[2] Jour. Med. Sc., 1894, 107, p. 31.
[3] Berl. klin. Wchnschr., 1899, 36, p. 638.
[4] Lancet, 1905, 2, p. 1760.
[5] Ztschr. f. d. ges. Neurol. u. Psychiat., O., 1910, 4, p. 91.
[6] Jour. Am. Med. Assn., 1914, 62, p. 110.
[7] Compt. Rend. Soc. de Biol., 1898, 5, p. 128.
[8] West. Lancet, 1883, 12, p. 531.
[9] Centralbl. f. Bakteriol., I, O., 1910, 52, p. 99.
[10] Am. Jour. Dis. Child., 1916, 12, p. 61.

producing long chain coccus in one culture from a patient who had a complicating endocarditis.

In one case Collins[11] isolated a coccus growing in pairs and short chains.

LaFetra[12] reports many blood cultures of chorea in Bellevue Hospital, New York, only 2 of which were positive, both yielding Streptococcus viridans.

Bartley[13] reports 4 negative cultures in 4 marked cases, and Koplik[14] reported many cases cultured with negative results.

A few observations on the bacteriology of the spinal fluid and central nervous system after death have been made; in practically all the cases diplococci were found. There is very little in the literature on the bacteriology of the cerebrospinal fluid in chorea during life.

Donath[5] isolated Staphylococcus aureus in one case.

Collins[11] reported negative results in all the cases he examined, and Passini[15] made observations on 5 patients with only negative results.

Morse and Floyd[10] cultured the spinal fluid of 19 patients, with negative results in all instances.

As to the tonsils, suffice it to say that Dick and Rothstein[16] isolated hemolytic streptococci from the throats of patients sick with chorea, and with one strain produced what seemed like chorea, in 12 hours, in a dog.

The results of animal experimentation appear to have been inconclusive. Only a few isolated experiments have been reported in which choreiform movements have been produced in animals. From observation of healthy rabbits and dogs, it may be concluded that only those animals that exhibit persistent and marked twitchings and tremors after injections may be considered as having any significance, because many normal young dogs and rabbits normally show irregular slight twitchings of the muscles of the limbs and face.

Pianese[1] produced tremor in dogs and rabbits by injection of cultures of the bacillus isolated from the blood of a case of chorea after death.

Poynton and Payne[4] describe twitchings, arthritis, and endocarditis in rabbits injected intravenously with diplococci obtained from the cerebrospinal fluid of a patient who died of chorea and rheumatism.

Donath[5] reported inconclusive results in animals injected with Staphylococcus albus and aureus obtained by blood culture of patients sick with chorea.

The experimental chorea described by Dick and Rothstein[16] in the dog is noteworthy, but as yet a preliminary report only of their work is at hand.

With the improvements in the technic in blood and tissue cultures in the living, in the past two decades, a large amount of work has been described which indicates a close association between hitherto unobserved foci of infection and acute infectious processes in remote parts of the body. Rosenow[17] and others have produced arthritis and endocarditis in rabbits by the intravenous injection of organisms obtained by culture of the tonsils, blood, and joints of patients with rheumatic

[11] Brit. Med. Jour., 1913, 1, p. 220.
[12] Arch. Pediat., 1915, 32, p. 135.
[13] Ibid., p. 137.
[14] Ibid., p. 561.
[15] Wien. klin. Wchnschr., 1914, 27, p. 1363.
[16] Jour. Am. Med. Assn., 1913, 61, p. 1376.
[17] Jour. Infect. Dis., 1914, 14, p. 61.

fever or endocarditis. Inasmuch as chorea is frequently associated with, or follows these conditions, I undertook to make cultures from the tonsils, and whatever other foci were observed, and from the blood and cerebrospinal fluid of acute, subacute and chronic cases of chorea, care being exercised to select only patients showing no evidence of active heart or joint involvement.

All instruments were boiled 10 minutes, and used directly from the sterilizer rack. The skin was washed with ether, painted with tincture of iodin, and allowed to dry. The blood was drawn from the median basilic vein, and transferred, after removal of the needle, to citrate solution, and inoculated within 15 minutes.

The spinal fluid was allowed to flow through the ordinary spinal puncture needle into sterile tubes, the end of the needle being flamed after removal of the obturator, and before collection of the fluid. In this case also cultures were made within 15 minutes.

Inoculations of the blood and cerebrospinal fluid were made in bottles of dextrose broth, anaerobic and aerobic tubes of ascitic dextrose broth, ascitic fluid rabbit kidney medium with paraffin oil layer, ascitic dextrose agar, ascitic dextrose blood-agar shake tubes, and blood-agar plates. Cultures were incubated at 37 C. for 10 days, and studied at intervals.

Material expressed from the tonsils, or swabs of the throat were streaked and plated on blood agar and kept under anaerobic and aerobic conditions.

Blood cultures were made in 21 patients, 10 of whom gave positive results. Nine gave small cocci, slightly elongated and arranged in pairs, short chains, and a few groups, in aerobic cultures. One only was strictly anaerobic. In 3 instances subcultures were sterile. Forty-eight hour broth cultures were slightly turbid, and there was a scant fine white sediment. On blood agar (10% goat blood) growth occurred in 1-4 days as fine colorless pin-point moist colonies. Two produced green about the colonies. None were hemolytic. Two strains grew in gelatin as very fine whitish colonies; the gelatin was not liquefied. In milk, 2 produced acid, but did not cause coagulation.

Of 6 strains of the cocci examined, all fermented dextrose and maltose, 5 fermented saccharose and lactose, 4 fermented mannite, 1 inulin, 1 raffinose, and 1 salicin.

The other positive blood culture gave a gram-positive short diphtheroid organism.

The cerebrospinal fluid of 21 patients has been cultured, with 13 positive results. One was not subcultured. Eight of the organisms isolated resembled very closely the organisms found in the blood. They were small, slightly elongated cocci, growing in pairs, short chains, and groups. Their growth on blood agar, gelatin, and in fluid mediums,

and their fermentation reactions, were in all respects similar to those of the cocci from the blood.

The other 5 organisms grew more luxuriantly in broth. Groups predominated, but there were a few in pairs, and a few short chains. On blood agar the colonies were larger, white, and more luxuriant after 24 hours. Their fermentative reactions were similar to those previously described.

Of 12 strains from the spinal fluid examined, all fermented dextrose, 11 maltose, 10 lactose, 9 saccharose, 3 mannite, 1 raffinose, and 1 inulin.

Throat cultures were made in 15 patients, and 29 cultures were isolated and studied.

Ten were hemolytic streptococci of variable size, occurring in long and short chains. Fermentation tests of 6 strains showed that all fermented raffinose and inulin, and 4 fermented saccharose.

Thirteen were green producing cocci, growing in pairs and short chains. Of 7 strains studied, all fermented raffinose, 6 inulin, 5 saccharose, and 3 dextrose.

Six were organisms which grew on blood agar as pin-point colorless nonhemolytic colonies, and resembled the organisms recovered from the blood in their morphologic and cultural characteristics, with the exception of their fermentative reactions. All fermented raffinose, 5 inulin, 3 saccharose, 3 dextrose, and 1 maltose.

The cases of chorea studied varied in duration from 10 days to 7 years, and in intensity from children who exhibited only mild choreiform movements in excitement to those so severe as to require restraint and feeding.

Those patients who yielded positive blood cultures had been ill from 10 days to 4 weeks; some, however, had recurring attacks.

Those yielding positive cultures from the cerebrospinal fluid had been sick from 10 days to 4 weeks, with the exception of one patient who had been ill 8 months.

Eleven patients gave histories of acute tonsillitis, two of rheumatism, and one of endocarditis. Enght gave no history of rheumatism, endocarditis, or tonsillitis.

A series of 25 rabbits were injected intravenously with various strains of organisms obtained from the tonsils, blood and spinal fluid. In no case were unmistakable persistent choreiform twitchings noted. In 2 rabbits there was some twitching of the forelegs and face 2 days and 12 hours, respectively, before death. A few animals succumbed in 24 hours, several in 5-7 days, and many

appeared unaffected. Postmortem examinations showed slight hyperemia of the meninges and brain in a few instances, and endocarditis in one. Cultures of the blood and spinal fluid did not consistently yield the organism injected.

One young dog was injected intravenously twice, the interval being 7 days, with an organism obtained from the cerebrospinal fluid of a marked case of chorea. No change was noted.

RESULTS OF EXAMINATION OF THE CEREBROSPINAL FLUID

There are many reports, chiefly French, of the cell count in the spinal fluid of patients ill with chorea. The majority of investigators have found a lymphocytosis.

Morse and Floyd[10] examined the fluid in 10 instances and report a lymphocytosis in 3.

Twenty-one spinal fluids were examined in this study, and cell counts made within 15 minutes after withdrawal of the fluid.

TABLE 1

Results Obtained in the Examination of 21 Spinal Fluids

Number of Cases	Cells per Cubic Millimeter
5	3
3	6
3	4
3	2
2	5
2	0
1	1
1	7
1	10

The average count was 3-6 cells. There was no constant relation between the severity of the chorea, the cultural results, and the cell count of the spinal fluid.

In all cases the tests for globulin carried out according to the technic of Nonne and Noguchi gave negative results.

Twelve of the 21 spinal fluids were tested for a reducing agent. All decolorized Fehling's solution, and produced a fine brick-red precipitate.

Wassermann tests with 3 antigens were negative in all cases.

SUMMARY

From so small a series of cases no general conclusions may be drawn. It is of interest that in 21 patients with chorea in public institutions none gave any evidence of syphilis, which consequently seems not to be of any etiologic importance in the disease.

TABLE 2
SUMMARY OF CASES

Number of Case, Sex and Age	Duration of Illness	Character of Attack	Cultures of Blood	Cultures of Cerebrospinal Fluid	Cultures of Tonsils	Previous Disease	Source
1—F 13	2 months	Very mild	No growth	Cocci, many	Hemolytic streptococci; green producing cocci; diplococci	Tonsillitis, rheumatism, endocarditis	Cook County Hospital
2—F 12	Irregularly, 4 years	Mild	Few diplococci	No growth	Hemolytic streptococci; green producing cocci	Tonsillitis	Cook County Hospital
3—M 8	2 weeks	Mild	No growth	No growth	Green producing cocci	....	Cook County Hospital
4—F 11	2 weeks	Very severe	Diplococci, many	Diplococci, many	Hemolytic streptococci; green producing cocci; diplococci	Tonsillitis	Cook County Hospital
5—M 9	3 weeks	Severe	Diplococci, few	Cocci, many	Hemolytic streptococci; diplococci	Tonsillitis	Cook County Hospital
6—F 21	3 weeks	Mild	No growth	No growth	Green producing cocci; diplococci	Rheumatism	Cook County Hospital
7—F 13	4 weeks	Very mild	No growth	No growth	Hemolytic streptococci; green producing cocci; diplococci	Tonsillitis	Cook County Hospital
8—F 11	2 weeks	Severe	No growth	No growth	Green producing cocci; hemolytic streptococci	Tonsillitis	Cook County Hospital
9—F 9	4 weeks	Severe	Diplococci, few	Diplococci, many	Hemolytic streptococci; diplococci	Tonsillitis	Cook County Hospital
10—M 9	6 weeks	Very mild	Diplococci, few	Diplococci, few	Green producing cocci	....	Cook County Hospital
11—F 8	7 weeks	Very mild	Diplococci, few	Cocci, many	Green producing cocci; hemolytic streptococci	....	Cook County Hospital
12—F 6	2 weeks	Severe	No growth	Diplococci, few	....	Tonsillitis	Cook County Hospital
13—F 12	4 months	Mild	No growth	Cocci, many	....	....	Childrens' Memorial Hospital
14—M 11	3 weeks	Very mild	Diplococci, few	Cocci, many	....	....	Childrens' Memorial Hospital
15—F 8	1 week	Severe	Diplococci, few	Cocci, many	....	....	Cook County Hospital
16—M 17	2 weeks	Very severe	No growth	Diplococci, few	....	....	Cook County Hospital
17—M 14	4 weeks	Severe	Diphtheroid organism	Diplococci, few	....	....	Cook County Hospital
18—F 19	2 years	Mild	No growth	No growth	....	Tonsillitis	Cook County Hospital
19—M 10	2 weeks	Severe	Diplococci, few	Diplococci, few	Hemolytic streptococci; green producing cocci	....	Cook County Hospital
20—F 9	4 weeks	Mild	No growth	No growth	Hemolytic streptococci; green producing cocci	....	Childrens' Memorial Hospital
21—F 21	4 months	Severe	No growth	No growth	Green producing cocci	....	Cook County Hospital

There is nothing characteristic or peculiar in the spinal fluid of chorea. Accepting the presence of globulin in the spinal fluid, and an increase in the number of cells, as indication of irritation or inflammation of the meninges, these results indicate that there are no changes in the meninges in chorea as a rule.

Of 21 cases, 10 yielded positive cultures from both the blood and spinal fluid, and 14 in either one or the other. Six of the bacterial strains isolated from the tonsil, 8 from the spinal fluid, and 9 from the blood, were identical in their morphologic and cultural characteristics. It therefore seems that bacteria are of importance in chorea and that the coccus mentioned in the foregoing is most frequently present, judging from the results in this series as well as the work of others.·

As to the grouping of the organism, its slow, scant, pin-point growth on blood agar, the slight turbidity and fine granular sediment produced in broth, would seem to relate it with the streptococci. In the predominance of pairs, with few short chains, and a few irregular groups, when grown in broth, it resembles the streptococci also, and especially the streptococci or diplococci frequently found in rheumatic fever and endocarditis. It should be noted that the strains studied do not as a rule cause hemolysis on blood-agar plates. No definite grouping can be made on the basis of the fermentative reactions as observed.

So far experiments on animals with this coccus, which is being studied further, have been inconclusive.

THE USE OF LARGER QUANTITIES OF BLOOD SERUM IN THE WASSERMANN REACTION

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The use of large quantities of blood serum in performing the Wassermann reaction has been advocated by others. For some reason it has not received the attention nor support that it deserves. Bohan and Lynch[1] showed conclusively the superiority of large quantities over small quantities of blood serum.

The following report deals with 50 serums, 15 of which came from nonsyphilitic persons, and 35 from syphilitics under treatment.

Method.—The Wassermann method with human hemolytic system was used in all the tests. Human serum, complement, antigen, hemolytic amboceptor and blood corpuscles were diluted with physiologic salt solution so that 0.25 c.c. of diluted ingredient represented the test dose, which brought the total qantity in each test tube up to 1.25 c.c.

Antigen.—The antigen was alcoholic extract of human heart muscle. The largest quantity that was not anticomplementary was used as the test dose.

Hemolytic Amboceptor.—The blood serum of a rabbit that had been immunized against washed human blood corpuscles was used in doses of from 1 to 2 units. The term unit was applied to the smallest quantity of hemolytic serum which with 0.025 c.c. of complement dissolved the test dose of blood corpuscles in 1 hour.

Corpuscles.—Human corpuscles were well washed and were used in doses of 0.25 c.c. of a 2.5% suspension in physiologic salt solution.

Complement.—The blood serum of guinea-pigs was used in doses of 0.05, 0.025 and 0.0125 c.c.

The human serums were heated to 56 C. for 30 minutes. Six test tubes, 1, 2, and 3 as antigen tubes, and 1', 2', and 3' as control tubes, were used in each test. Each of the 6 tubes received 0.25 c.c. of diluted human serum. Each of the 1st pair of tubes, Tubes 1 and 1', received 0.25 c.c. of 1:5 dilution of complement serum; each of the 2nd pair of tubes, Tubes 2 and 2', received 0.25 c.c. of 1:10 dilution of complement serum; and each of the 3rd pair of tubes received 0.25 c.c. of 1:20 dilution of complement serum.

Each of the antigen tubes, Tubes 1, 2, and 3, received 0.25 c.c. of diluted antigen; and the control tubes, Tubes 1', 2', and 3', received 0.25 c.c. of physiologic salt solution each. These mixtures were placed in the incubator at 37 C. for 1 hour. After having been in the incubator 1 hour each tube received 0.5 c.c. of sensitized corpuscles representing 0.25 c.c. of 2.5% suspension of corpuscles and 0.25 c.c. of diluted hemolytic amboceptor which were mixed

[1] Jour. Am. Med. Assn., 1917, 69, p. 1220.

30 minutes before they were added to the contents of the tubes. After shaking, the tubes were returned to the incubator for another hour during which time they were shaken 3 or 4 times. After an hour in the incubator the tubes were allowed to stand at room temperature for 3 or 4 hours, after which the results were read and recorded.

TEST 1

Serums 1 to 10, inclusive, were obtained from nonsyphilitic persons. Within 24 hours after the bleeding these serums were tested in quantities of 0.05 c.c. and 0.25 c.c. per test tube.

TABLE 1

Wassermann Reaction with Varying Quantities of Fresh Nonglycerolated Human Serum

No. of Serum	Quantity of Serum per Tube C.c.	Quantity of Amboceptor per Tube Unit	Readings*						Results
			Antigen Tubes			Control Tubes			
			1	2	3	1′	2′	3′	
1	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
2	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
3	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
4	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
5	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
6	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
7	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
8	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
9	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
10	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative

* Explanation: tr = hemolysis less than 50 %; ± = hemolysis between 50 and 100 %; + = complete hemolysis.

Table 1 shows the results obtained with the first 10 serums. The larger quantities of serum gave results identical with those obtained with the smaller quantities and were but slightly anticomplementary. These larger quantities did not give false positive results.

TEST 2

Serums 11 to 15 inclusive, were obtained from nonsyphilitic persons and serums 16 to 20, inclusive, came from syphilitics under treatment.

Each serum was divided into 2 portions, A and B. Portion A remained untreated and Portion B was mixed with an equal volume of sterilized Merck's blue label glycerol. Each portion was tested in quantities of 0.05 c.c. and 0.125 c.c. of serum (0.1 c.c. and 0.25 c.c. of serum-glycerol mixture) per test tube.

TABLE 2

WASSERMANN REACTION WITH VARYING QUANTITIES OF FRESH NONGLYCEROLATED AND GLYCEROLATED HUMAN SERUM

No. of Serum	Portions: A = Nongly-cerated, B = Gly-cerated	Quantity of Serum per Tube, C.c.	Quantity of Ambo-ceptor per Tube Unit	Antigen Tubes			Control Tubes			Results	
				1	2	3	1′	2′	3′		
11	A	0.05	1.0	+	+	±	+	+	±	Negative	
	A	0.125	1.0	+	+	±	+	+	±	Negative	
	B	0.05	1.25	+	+	±	+	+	±	Negative	
	B	0.125	1.5	+	+	±	+	+	±	Negative	
12	A	0.05	1.0	+	+	±	+	+	±	Negative	
	A	0.125	1.0	+	+	±	+	+	±	Negative	
	B	0.05	1.25	+	+	±	+	+	±	Negative	
	B	0.125	1.5	+	+	±	+	+	±	Negative	
13	A	0.05	1.0	+	+	±	+	+	±	Negative	
	A	0.125	1.0	+	+	±	+	+	±	Negative	
	B	0.05	1.25	+	+	±	+	+	±	Negative	
	B	0.125	1.5	+	+	±	+	+	±	Negative	
14	A	0.05	1.0	+	+	±	+	+	±	Negative	
	A	0.125	1.0	+	+	±	+	+	±	Negative	
	B	0.05	1.25	+	+	±	+	+	±	Negative	
	B	0.125	1.5	+	+	±	+	+	±	Negative	
15	A	0.05	1.0	+	+	±	+	+	±	Negative	
	A	0.125	1.0	+	+	±	+	+	±	Negative	
	B	0.05	1.25	+	+	±	+	+	±	Negative	
	B	0.125	1.5	+	+	±	+	+	±	Negative	
16	A	0.05	1.0	+	+	±?	+	+	±	Faintly positive,	±
	A	0.125	1.0	+	+	tr	+	+	±	Weakly positive,	+
	B	0.05	1.25	+	+	tr	+	+	±	Weakly positive,	+
	B	0.125	1.5	+	±	0	+	+	±	Strongly positive,	+++
17	A	0.05	1.0	+	+	±?	+	+	±	Faintly positive,	±
	A	0.125	1.0	+	+	tr	+	+	±	Weakly positive,	+
	B	0.05	1.25	+	+	tr	+	+	±	Weakly positive,	+
	B	0.125	1.5	+	±	0	+	+	±	Strongly positive,	+++
18	A	0.05	1.0	+	+	tr	+	+	±	Weakly positive,	+
	A	0.125	1.0	+	+	0	+	+	±	Moderately positive,	++
	B	0.05	1.25	+	+	0	+	+	±	Moderately positive,	++
	B	0.125	1.5	+	tr	0	+	+	±	Strongly positive,	++++
19	A	0.05	1.0	+	+	tr	+	+	±	Weakly positive,	+
	A	0.125	1.0	+	+	0	+	+	±	Moderately positive,	++
	B	0.05	1.25	+	+	tr	+	+	±	Weakly positive,	+
	B	0.125	1.5	+	tr	0	+	+	±	Strongly positive,	++++
20	A	0.05	1.0	+	+	0	+	+	±	Moderately positive,	++
	A	0.125	1.0	+	±	0	+	+	±	Strongly positive,	+++
	B	0.05	1.25	+	+	0	+	+	±	Moderately positive,	++
	B	0.125	1.5	±	0	0	+	+	±	Strongly positive,	++++++

* Explanation: 0 = no hemolysis; tr = hemolysis less than 50 %; ± = hemolysis between 50 and 100 %; + = complete hemolysis.

Table 2 shows that serums 11 to 15, inclusive, gave negative results with all of the four methods. Serums 16 to 20, inclusive, gave positive results. The larger quantities of serum gave stronger positive results than did the smaller quantities, and the glycerolated portions gave stronger positive results than did the nonglycerolated portions. This was particularly true of the larger quantities of the glycerolated portions, which reacted much stronger than did corresponding quantities of the nonglycerolated portions. Glycerol being anticomplementary, the larger quantities required larger quantities of hemolytic amboceptor.

TEST 3

Thirty old glycerolated serums were tested in quantities of 0.05 and 0.125 c.c. per test tube.

TABLE 3

Wassermann Reaction with Varying Quantities Old Glycerolated Human Serum

No. of Serum	Quantity of Serum per Tube, C.c.	Quantity of Amboceptor per Tube, Unit	Readings*						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
21	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	tr	+	+	tr	Negative	
22	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	tr	+	+	tr	Negative	
23	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	tr	+	+	tr	Negative	
24	0.05	1.5	+	+	±?	+	+	±	Faintly positive,	±
	0.125	2.0	+	+	0	+	+	tr	Weakly positive,	+
25	0.05	1.5	+	+	±?	+	+	±	Faintly positive,	±
	0.125	2.0	+	+	0	+	+	tr	Weakly positive,	+
26	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	0	+	+	tr	Weakly positive,	+
27	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	tr	+	+	tr	Negative	
28	0.05	1.5	+	+	tr	+	+	±	Weakly positive,	+
	0.125	2.0	+	±	0	+	+	tr	Moderately positive,	++
29	0.05	1.5	+	+	0	+	+	±	Moderately positive,	++
	0.125	2.0	+	0	0	+	+	tr	Strongly positive,	++++
30	0.05	1.5	+	+	0	+	+	±	Moderately positive,	++
	0.125	2.0	+	0	0	+	+	tr	Strongly positive,	++++
31	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	tr	+	+	±	Weakly positive,	+
32	0.05	1.5	+	±	tr	+	+	tr	Faintly positive,	±
	0.125	2.0	+	+	tr	+	+	±	Weakly positive,	+

* Explanation: 0 = no hemolysis; tr = hemolysis less than 50 %; ± = hemolysis between 50 and 100 %; + = complete hemolysis.

TABLE 3—*Continued*

WASSERMANN REACTION WITH VARYING QUANTITIES OLD GLYCEROLATED HUMAN SERUM

No. of Serum	Quantity of Serum per Tube, C.e.	Quantity of Ambo-ceptor per Tube, Unit	Readings*						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1′	2′	3′		
33	0.05	1.5	+	+	tr	+	+	tr	Negative	
	0.125	2.0	+	+	tr	+	+	±	Weakly positive,	+
34	0.05	1.5	+	+	tr	+	+	tr	Negative	
	0.125	2.0	+	±	0	+	+	0	Weakly positive,	+
35	0.05	1.5	+	+	tr	+	+	tr	Negative	
	0.125	2.0	+	+	tr	+	+	±	Weakly positive,	+
36	0.05	1.5	+	+	tr	+	+	tr	Negative	
	0.125	2.0	+	tr	0	+	+	tr	Strongly positive,	+++
37	0.05	1.5	+	+	tr?	+	+	tr	Faintly positive,	±
	0.125	2.0	±	0	0	+	+	tr	Strongly positive,	+++++
38	0.05	1.5	+	+	0	+	+	tr	Weakly positive,	+
	0.125	2.0	tr	0	0	+	+	tr	Strongly positive,	++++++
39	0.05	1.5	+	+	0	+	+	tr	Weakly positive,	+
	0.125	2.0	0	0	0	+	+	tr	Strongly positive,	+++++++++
40	0.05	1.5	+	±	0	+	+	tr	Moderately positive,	±+
	0.125	2.0	0	0	0	+	+	tr	Strongly positive,	+++++++++
41	0.05	1.5	+	+	0	+	+	tr	Weakly positive,	+
	0.125	2.0	+	tr	0	+	+	tr	Strongly positive,	+++
42	0.05	1.5	+	tr	0	+	+	tr	Strongly positive,	+++
	0.125	2.0	0	0	0	+	+	0	Strongly positive,	++++++
43	0.05	1.5	+	0	0	+	+	tr	Strongly positive,	++++
	0.125	2.0	0	0	0	+	+	tr	Strongly positive,	++++++++
44	0.05	1.5	+	tr	0	+	+	tr	Strongly positive,	+++
	0.125	2.0	0	0	0	+	+	tr	Strongly positive,	+++++++++
45	0.05	1.5	+	tr	0	+	+	tr	Strongly positive,	+++
	0.125	2.0	tr	0	0	+	+	tr	Strongly positive,	++++++
46	0.05	1.5	+	±	0	+	+	tr	Moderately positive,	++
	0.125	2.0	tr	0	0	+	+	tr	Strongly positive,	++++++
47	0.05	1.5	+	±	0	+	+	tr	Moderately positive,	++
	0.125	2.0	0	0	0	+	+	tr	Strongly positive,	++++++++
48	0.05	1.5	+	tr	0	+	+	tr	Strongly positive,	+++
	0.125	2.0	0	0	0	+	+	tr	Strongly positive,	++++++++
49	0.05	1.5	+	tr	0	+	+	tr	Strongly positive,	+++
	0.125	2.0	0	0	0	+	+	tr	Strongly positive,	++++++++
50	0.05	1.5	+	0	0	+	+	tr	Strongly positive,	++++
	0.125	2.0	0	0	0	+	+	tr	Strongly positive,	++++++++

* Explanation: 0 = no hemolysis; tr = hemolysis less than 50 %; ± = hemolysis between 50 and 100 %; + = complete hemolysis.

The results are shown in Table 3. The larger quantities of serum gave much stronger positive results than did the smaller quantities. With Serums 36, 37, and 39 the differences were unusually marked. Of Serum 36 the smaller quantity gave a negative result, and the larger

quantity gave a strongly positive result. Serum 37 gave faintly positive ($\pm$) and strongly positive ($+++++$) results, while Serum 39 gave $+$ and $++++++++$. These results are slightly out of proportion because the larger quantities of serum were only $2\frac{1}{2}$ times as large as the smaller quantities.

CONCLUSIONS

Of fresh serum quantities amounting to 0.2 of the total volume per test tube did not give false positive results and were but slightly anti-complementary.

With larger quantities of serum a higher percentage of positive results is obtained.

Glycerol is anticomplementary; the anticomplementary action may be overcome by increasing the quantity of amboceptor.

Unconditional negative results should not be reported unless fairly large quantities of serum were used in the test.

THE TREATMENT OF SCARLET FEVER WITH IMMUNE HUMAN SERUM

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All efforts to identify and isolate the specific cause of scarlet fever having failed, it has been impossible to produce an immune serum for therapeutic purposes in this disease similar to that employed in the treatment of tetanus, diphtheria, epidemic meningitis and pneumonia. However, it was naturally inferred that persons who had recently passed through an attack of scarlet fever would have specific antibodies in their blood and, should the antibodies be in sufficient concentration, that the serum of such blood might be of curative value when introduced into patients acutely sick with the disease.

Acting on this presumption, Weisbecker,[1] in 1897, treated 5 cases of scarlet fever with the blood serum of convalescents, but with little success. From 1897 to 1903, scarlet fever cases were treated by injections of convalescent serum by Huber and Blumenthal,[2] von Leyden,[3] Rumpel[4] and Scholz.[5] They did not reach any certain conclusion as to the value of the procedure. The serum was injected subcutaneously and in relatively small doses. Because of the absence of any decided advantage from this treatment and from the fear of transmitting syphilis and other infections the use of convalescent serum was abandoned for about 10 years. By this time the Wassermann test made it possible to exclude syphilis from the donor of the serum, and a large experience in the administration of large quantities of serums had been acquired. Intravenous injections of serum had also been successfully employed in various cases. In 1912, Reiss and Jungmann[6] treated 12 cases of severe scarlet fever by intravenous injections of 40 to 100 c.c. of convalescent serum with marked benefit in 10 cases. They drew the blood from convalescents about the end of the 3rd or beginning of the 4th week of the disease, tested each serum for syphilis and sterility,

[1] Ztschr. f. klin. Med., 1897, 32, p. 188.
[2] Berl. klin. Wchnschr., 1897, 34, p. 671.
[3] Deutsch. Arch. f. klin. Med., 1902, 73, p. 616.
[4] München. med. Wchnschr., 1903, 50, p. 38.
[5] Fortschr. d. Med., 1903, 21, p. 353.
[6] Deutsch. Arch. f. klin. Med., 1912, 106, p. 70.

and mixed the serums from several persons, since some persons seemed to yield larger amounts of antibodies than others. The next year Koch[7] reported from the same clinic the treatment of 22 additional patients with 1 death. He emphasized the value of early administration. He noted a prompt fall in fever usually followed by a slight rise, and he was especially impressed by the rapid and marked improvement in the general condition of the patient. In 1915, Reiss and Hertz,[8] and Koch[9] reported still larger series of cases in which the results were most satisfactory.

In April, 1915, Zingher[10] reported the treatment of scarlet fever with fresh blood from convalescents. In the fall of 1915[11] he reported the treatment of 15 cases of very severe scarlet fever, selected from 900 cases, with 4 deaths. Two of the patients died from septic conditions, and the other 2 patients who died were moribund when they were received. Of the 11 patients who recovered, 5 were of the purely toxic type, the other 6 had additional septic complications. The 5 purely toxic cases showed a critical drop in temperature after which it remained normal or slightly above normal. In the 6 remaining cases the drop in temperature was less marked and was followed by a secondary rise which persisted for a number of days. Zingher has departed from the technic of the earlier writers in that he has substituted the intramuscular injection of citrated blood for the intravenous injection of serum. The blood is drawn from the median cephalic vein at the bend of the elbow and immediately citrated by adding 1 c.c. of 10% solution of sodium citrate to each 30 c.c. of blood. This fresh citrated blood is injected intramuscularly, introducing 15-30 c.c. in each of several larger muscular masses. Of this blood 75-240 c.c. are given at a dose. The blood is rapidly absorbed.

During the past winter and spring an unusual proportion of severe cases of scarlet fever has come under treatment in the Durand Hospital, and the most severe were selected for treatment with convalescent serum. The blood was drawn from the 20th-28th day, only such convalescents being selected as were free from all suspicions of tuberculosis, who had not been septic patients, and who gave a negative Wassermann reaction. The serums from several patients were mixed, tested for sterility, and stored, until used, in the refrigerator in

[7] München. med. Wchnschr., 1913, 60, p. 2611.

[8] Ibid., 1915, 62, p. 1177.

[9] Deutsch. med. Wchnschr., 1915, 41, p. 372.

[10] New York State Jour. Med., 1916, 16, p. 112.

[11] Jour. Amer. Med. Assn., 1915, 65, p. 875.

portions suitable for single doses. The serum was injected intramuscularly in doses of 25-90 c.c., 60 c.c. being the usual dose. Commonly a single dose was given, a few times a second dose was administered if the initial one did not produce the desired effect. The injections were made into the muscles on the outer side of the thigh, usually dividing the dose between the two sides. No local or general disturbances followed the injections.

Nineteen cases of scarlet fever were treated in this manner and in a few additional cases convalescent serum was combined with antidiphtheria serum in cases of scarlet fever complicated by diphtheria. Since it is more difficult to judge of the value of the convalescent serum in the latter cases they will not be discussed here except to state that all recovered although some seemed almost hopeless.

The effects of injections of convalescent serum in cases of scarlet fever as observed by me correspond to those noted by others who have used it. Quite constantly a fall of temperature began 2-4 hours after the serum was injected and continued gradually until its limit was reached in 12-24 hours. In the purely toxic cases the temperature fell to nearly normal and showed little tendency to rise again. In cases with septic complications the initial fall of temperature also occurred and was associated with general improvement, but the fever rose again and ran a course such as occurs in septic cases. However, in these cases the fever did not often rise to its original height, and the course of the disease appeared to be favorably altered by the serum. During the 12-24 hours after the injection of the serum, more striking than the fall in temperature, was the improvement in the general condition of the patient. This was most pronounced in the purely toxic cases, but also very prominent in the septic ones. Delirium subsided, the pulse became slower and improved in quality, cyanosis disappeared, and in 12-24 hours patients who had seemed almost hopelessly sick were apparently on the road to certain recovery. In 2 cases a fall of temperature and general improvement followed the injection of serum, but the fever rose again and the general condition became worse. A second injection of serum was followed by rapid improvement. Septic cases coming under treatment late do not appear to be improved by the serum.

It will be noted that most of the patients were received at the hospital on the 4th and 5th day of the attack, and that the patients who received the serum early were most greatly influenced. We have

thought that if the serum were given early some later septic complications might be avoided. It must not be forgotten that scarlet fever usually runs a relatively short course and that cases of the toxic type not infrequently terminate almost by crisis. The happy course taken by so many cases of severe scarlet fever as have now been reported as treated with convalescent serum can hardly be due to coincidence. To those accustomed to seeing large numbers of cases of scarlet fever, the general improvement in these patients within a few hours is entirely new and unexpected. The comparative value of serum and whole citrated blood must be determined, but except in large hospitals in which suitable convalescents are constantly available, the use of sterile serum which can be stored until a suitable case appears has a distinct advantage. This also allows several serums to be combined in each case. How long after recovery the blood contains sufficient antibodies to be of therapeutic value should be determined, as this would have considerable influence in determining the available supply of serum. Naturally the study of this whole subject is rendered difficult by the absence of an experimental scarlet fever in animals.

CASE 1.—Boy, age 10 years. A toxic and septic case admitted on the 5th day with a temperature of 104 F., slightly hemorrhagic eruption, ulcerative angina, eyes very red, active delirium, head retracted, involuntary urination, cyanosis, and profuse purulent nasal discharge. Sixty c.c. of convalescent serum were given, following which the temperature fell gradually to 101 F. in 12 hours, the general condition improved and was replaced by sleep. Some fever, between 100 and 101 F. continued for 2 weeks associated with otitis media and suppurating glands. Recovery.

CASE 2.—Girl, age 16 years. A toxic case admitted on the 4th day with a temperature of 104 F. and pulse of 150, active delirium, eyes red, moderate cervical glandular and periglandular swelling, ulcerative angina, slight nephritis, lips, skin and nails blue. Sixty c.c. of convalescent serum were given, and 12 hours later the temperature had fallen to 100.6 F., pulse to 120, and the general condition was very much improved. The delirium was much less but did not entirely disappear until 24 hours later. For 9 days a fever of 102 F. or less continued in association with a very profuse purulent nasal and pharyngeal discharge. Recovery.

CASE 3.—Woman, age 23 years (Chart 1). A toxic case admitted on the 4th day with temperature of 103 F. and pulse of 140, severe ulcerative tonsillitis, polyarthritis, severe bilateral cervical adenitis, slight nephritis, active delirium, cyanosis of skin, lips and nails. The next morning the condition was unimproved, and 60 c.c. of convalescent serum were given. In 12 hours the temperature had fallen to 99 F. and the pulse to 100, the mind was clear, and the general condition was entirely changed for the better. For 3 days the temperature rose to 90 F. and then was normal. Recovery.

CASE 4.—Boy, age 12 years. Severe toxic case admitted on the 4th day with a temperature of 104 F., a weak and irregular pulse of 150, severe angina, mild
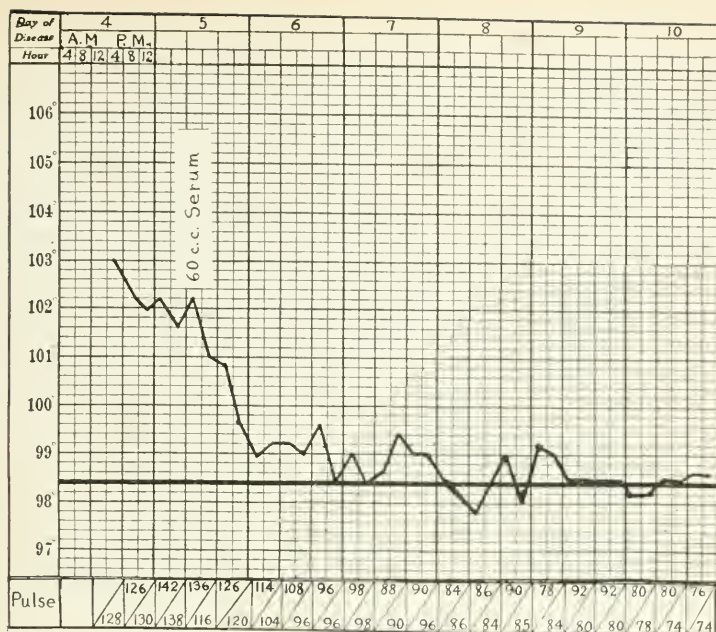
Chart 1.—Temperature curve, Case 3.
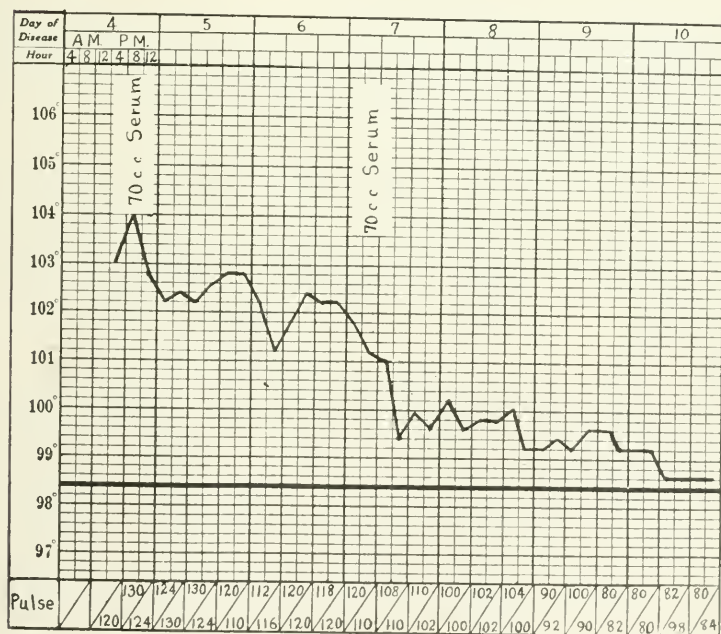
Chart 2.—Temperature curve, Case 6.

delirium, and cyanosis. Thirty-five c.c. of convalescent serum were given and the temperature fell in 12 hours to 102 F., with much improvement in the general condition. During the 5 following days the temperature gradually fell to normal. Recovery.

Case 5.—Man, age 27 years. A severe toxic case admitted on the 4th day with a temperature of 104 F., a weak and intermittent pulse of 130, ulcerative tonsillitis, severe parenchymatous nephritis, eyes red, slightly hemorrhagic eruption, epistaxis, low delirium, lying with eyes partially closed. Fifty c.c. of convalescent serum were given and 20 hours later the temperature had gradually fallen to 102 F. and a marked improvement had taken place in the general condition. A 2nd dose of 25 c.c. of serum was given and 20 hours later the temperature had fallen to 100 F. and the general condition of the patient was still more favorable. He was slightly irrational another day. Slight fever running up to 101 F. continued 7 days, associated with persistent severe angina. Recovery.

Case 6.—Man, age 19 years (Chart 2). A severe toxic case, admitted on the 4th day with a temperature of 104 F., pulse 130, partially irrational, and severe angina. Seventy c.c. of convalescent serum were given following which the temperature fell to 102 F. in 12 hours and the general condition seemed somewhat better. For 2 days the temperature ran about 102 F. and the patient became actively delirious, pulse weak and irregular and skin, lips and nails cyanotic. Seventy c.c. of serum were given and in the following 12 hours the temperature fell to 99.5 F., the delirium almost entirely disappeared, and the general condition improved enormously. For 2 days further the temperature was between 99 and 100 F. and then became normal. Recovery.

Case 7.—Woman, age 22 years (Chart 3). A toxic case, admitted on the 4th day with a temperature of 104.3 F., pulse 134 and slightly irregular, eyes red, severe headache and vomiting. Sixty c.c. of convalescent serum were given, following which the temperature began to gradually fall reaching 100 F. in 20 hours, and with this the general condition was much improved. The following day the temperature rose to 101 F. for a short time and again fell to 99.5 F. and was normal the next day. Recovery.

Case 8.—Girl, age 2½ years. A septic patient who entered the hospital on the 4th day with a temperature of 104 F., pulse of 146, dull mentally, marked bilateral cervical adenitis and periadenitis, ulcerative angina and stomatitis, vomiting, profuse purulent nasal discharge, and cyanosis. Thirty c.c. of convalescent serum were given following which the temperature fell to 100 F. in 12 hours, but it again rose to 105 F., when an additional 30 c.c. of serum were given but with no apparent effect. The temperature remained high and death occurred 4 days after entrance.

Case 9.—Man, age 26 years. A very toxic case, admitted on the 5th day with a temperature of 103 F., headache, severe angina, nausea, subconjunctival hemorrhage, bloody discharge from nose, multiple arthritis, and talkative delirium. For 2 days the condition grew worse, when on the 7th day of illness, 50 c.c. of convalescent serum were given. The temperature soon began to fall and in 20 hours had dropped from 103 to 100 F., and simultaneously the delirium disappeared and the general condition improved very much. The temperature ran about 100 F. for two days and became normal on the 13th day of illness or 6 days after the serum was injected. Recovery.

Case 10.—Boy, age 6 years. Moderately severe toxic case, admitted on the 3rd day with severe angina, vomiting, and reddened eyes. Twenty-five c.c. of convalescent serum were given, following which the temperature fell from
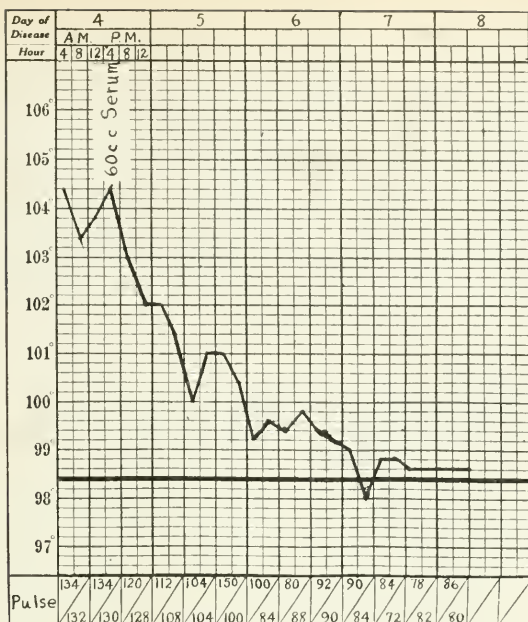
Chart 3.—Temperature curve, Case 7.
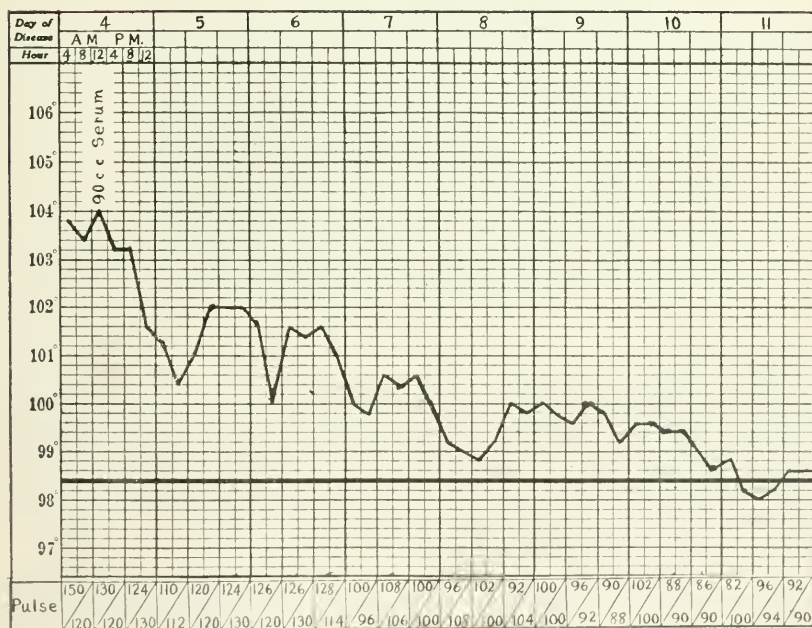
Chart 4.—Temperature curve, Case 12.

103 to 100.2 F. in 20 hours. Twelve hours later it had risen again to 101.6 F. when it again began to fall and was normal on the 8th day. Recovery.

CASE 11.—Boy, age 4½ years. Severe toxic case, admitted on the 2nd day of illness. During the following day the fever ran about 104 F. with pulse of 150, eyes red, nausea, severe angina, active delirium, profuse purulent nasal discharge, and moderate cervical adenitis. Sixty-eight c.c. of convalescent serum were given on the 4th day. A few hours later the general condition began to improve but no immediate drop of temperature occurred. Twenty-four hours later after the serum was given the temperature, which had remained about 104 F., began to fall and reached 99 F. 2 days later and was normal on the 8th day. Recovery.

Chart 5.—Temperature curve, Case 17.

CASE 12.—Woman, age 26 years (Chart 4). Severe toxic case admitted on the 4th day with a temperature of 104 F., pulse 150, eyes red, severe angina, and moderate delirium. Ninety c.c. of convalescent serum were given, following which the temperature fell to 100.4 F. in 20 hours, and the general condition improved greatly. The following day the temperature rose a short time to 102 F. and then gradually became less, being normal on the 10th day. The very rapid improvement in the general condition was much more marked than was the effect on the temperature. Recovery.

CASE 13.—Woman, age 23 years. Severe toxic case, admitted on the 3rd day with a temperature of 104.2 F., and a pulse of 140. The following day the temperature was 104.5 F., pulse 160, severe ulcerative angina, headache, persistent nausea and vomiting. Ninety c.c. of convalescent serum were given and in 20

hours the temperature was 101 F. with great improvement in the general condition. There were temporary rises of temperature to 102.5 and 102 F. on the 2 following days, but 1 day later the temperature became permanently normal. Recovery.

Case 14.—Boy, age 7 years. Moderately severe toxic case with septic complications, admitted on the 3rd day with a temperature of 103 F. The following day the patient was worse, temperature 104 F., pulse 150, headache, vomiting, polyarthritis, and purulent discharge from left ear. Sixty-five c.c. of convalescent serum were given, following which the temperature fell to 100.5 F. in 20 hours and the general condition was much improved. The temperature ran from 100.5 to 102 F. for 2 days associated with double purulent otitis media and polyarthritis. From the 8th day the temperature was normal. Recovery.

Case 15.—Girl, age 11 years. Rather severe toxic case, admitted on the 5th day with a temperature of 104 F. Sixty c.c. of convalescent serum were given and the temperature fell to 99.6 in 24 hours. For five days the temperature ran from 99.6-101 F., then became normal. Recovery.

Case 16.—Girl, age 8 years. A very severe toxic case, admitted on the 2nd day with a temperature of 104.2 F., pulse 160, moderate angina, and active delirium. Sixty c.c. of convalescent serum were given, following which the temperature fell to 100 F. in 20 hours; the patient slept quietly and the general condition was greatly changed for the better, an almost hopeless condition being replaced by a very favorable one. The remaining fever gradually subsided, reaching normal after 3 days. At this time a very severe stomatitis developed with which the temperature again rose to 101.8 F. and continued 4 days. On the 10th day the temperature became permanently normal. Recovery.

Case 17.—Girl, age 4 years (Chart 5). A toxic case of moderate severity admitted on the evening of the second day. During the following 24 hours the temperature ran from 102.5-103.8 F., pulse 140-160, general toxic symptoms severe, but without septic complications. Now 60 c.c. convalescent serum were given, following which the temperature dropped to 99.2 F. in 12 hours and to 98.2 F. in 16 hours, and did not rise above normal subsequently. Recovery.

Case 18.—Girl, age 5 years. A toxic case admitted on the evening of the 3rd day with a temperature of 105 F. During the night the patient had repeated convulsions, high fever, and delirium. On the morning of the 4th day, 35 c.c. of convalescent serum were given. Soon the temperature began to fall and gradually subsided to 99 F. in 24 hours, associated with very marked improvement in the general condition. The temperature did not again rise above 99.4 F. and was normal after the 9th day of illness. Recovery.

Case 19.—Girl, age 4 years. A septic case admitted on the 7th day with profuse purulent nasal discharge, ulcerative stomatitis and polyarthritis. Sixty c.c. of convalescent serum were given with no apparent effect on the septic process. During the 16 hours after the serum was given the temperature fell from 103 to 99 F. but rose again to the original height in the succeeding 16 hours, and remained between 101.5 and 103 F. for 3 days, then gradually subsided. Recovery.

A CONTRIBUTION TO THE PROPHYLAXIS OF
LOBAR PNEUMONIA

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While the earliest investigations in the bacteriology of pneumonia have shown the presence of pneumococci in the mouth secretions of healthy persons as well as those suffering with lobar pneumonia, recent investigations show that the disease is frequently acquired by transmission of specific virulent types of pneumococci from a recovered person who still harbors in the mouth-secretions the organisms responsible for the disease, or from a healthy carrier of pathogenic pneumococci. Dochez and Avery[1] found about 40% of healthy persons intimately associated with cases of lobar pneumonia harboring the same types of pneumococci as those producing the disease; these carriers included physicians, nurses, and members of the patients' families as wives, mothers, brothers and sisters. Convalescents from pneumonia were found to carry the type of pneumococcus with which they have been infected, for a considerable length of time — in one instance as long as 90 days, counting from the first day of illness.

Stillman[2] found pneumococci in the mouths of 172 normal persons of 398 studied; in 8 of these persons Types I and II pneumococci were present, while 26 persons harbored typical Type II organisms and 44 Type III. Of 84 persons studied who had been in contact with cases of pneumonia, 5, or 5.95%, showed either pneumococci of Type I or Type II in their sputum; the shortest period of carrying was 7 days, the longest 85 days. Sydenstricker and Sutton[3] found from 6-22% of healthy persons harboring pneumococci of the fixed types in the sputum and about 32% harboring the ordinary saprophytic Type IV organisms. In a more recent summary of studies of a large number of healthy individuals in contact with cases of lobar pneumonia due to Type I and

[1] Jour. Exper. Med., 1915, 22, p. 105.
[2] Ibid., 1916, 24, p. 651.
[3] Bull. Johns Hopkins Hospital, 1917, 28, p. 312.

Type II pneumonococci, Avery, Chickering, Cole and Dochez[4] report about 12% were found to carry the corresponding types of pneumococci, whereas among healthy persons not in contact with cases of lobar pneumonia, these types were found in but 0.3 or less per cent.

These investigations having definitely established the existence of the carrier state of disease producing pneumococci among healthy persons and those recently recovered from pneumonia, and indicating the means by which lobar pneumonia may be spread, it would appear advisable to attempt the destruction of pneumococci in the mouth secretions particularly among convalescents and those in intimate contact with cases of lobar pneumonia. We have approached this problem mainly from the laboratory side bearing in mind the numerous failures which have followed clinical attempts to disinfect mucous membranes and secretions of various bacteria.

As the investigations of Morgenroth and Levy,[5] Wright,[6] Moore,[7] Cohen, Kolmer and Heist[8] have shown the high pneumococcidal activity of cinchona derivatives and particularly ethylhydrocuprein, we have made our studies on the disinfection of sputum and the mouth with solutions of various cinchonics in a menstruum of liquor thymolis [*] our experiments demonstrating that the latter solution alone possesses some germicidal activity for pneumococci and aids in disguising the bitter taste of cinchona compounds. Wadsworth[9] has tested the power of saline solutions and bland alcoholic washes in ridding the mouth of pneumococci, but no specific pneumococcidal substances were used; his results, however, were rather encouraging.

In our experiments both normal mouth secretions harboring Type IV pneumococci and the sputum of pneumonia convalescents harboring Type I organisms were employed and the pneumococcidal activity of the substance under experiment determined largely by mouse inoculation.

Preliminary mouse inoculation tests with the mouth secretions of several normal persons who had not recently been in contact with cases of pneumonia showed that the intraperitoneal injection of 1 c.c. of their sputum produced

[4] Monographs of the Rockefeller Institute, 1917, No. 7, Acute Lobar Pneumonia.

[5] Berl. klin. Wchnschr., 1911, 48, pp. 1561, 1650, 1779, 1983.

[6] Lancet, 1912, 11, pp. 1633, 1701.

[7] Jour. Exper. Med., 1915, 22, p. 269.

[8] Jour. Infect. Dis., 1917, 20, p. 272.

[*] Liquor thymolis used in the Polyclinic hospital as a substitute for liquor antisepticus, is prepared as follows: benzoic acid, 64 grains; boric acid 128 grains; thymol and menthol, each 16 grains; oil of eucalyptus, oil of wintergreen and oil monarda each 4 drops; alcohol and glycerin, each 4 ounces; water sufficient for 16 ounces.

[9] Ibid., 1906, 3, p. 774.

death in about 24 hours with Type IV pneumococci in the peritoneal cavity and blood of the heart; the injection of 0.25-0.5 c.c. of sputum killed at irregular intervals but usually within 4 days.

Experiments conducted with a centrifuge method previously described by Kolmer[10] yielded results as shown in Table 1. In conducting these tests the mouth secretions of one or more normal persons were collected in sterile vials and 1 c.c. placed in a series of sterile centrifuge tubes with 1 c.c. of the varying dilutions of a cinchonic made up with liquor thymolis; after thorough mixing, the tubes were incubated at 35 C. for 10 minutes, thoroughly centrifuged and the supernatant fluids discarded. To the sediment in each tube 1.5 c.c. of sterile salt solution was added and 1 c.c. injected intraperitoneally in mice and 0.2 c.c. plated with blood agar.

TABLE 1

THE GERMICIDAL ACTIVITY OF VARYING DILUTIONS OF CINCHONICS IN 1:4 LIQUOR THYMOLIS ON TYPE IV PNEUMOCOCCI IN MOUTH SECRETIONS OF NORMAL PERSONS (CENTRIFUGE TESTS)

Cinchonic	Final Dilutions of Cinchonic	Animal Tests			Plates 48 Hour Counts
		24 Hour	48 Hour	72 Hour	
Ethylhydrocuprein	1:10,000	S*	S	S	5,700
	1:16,000	S	S	S	7,900
	1:20,000	S	S	S	Uncountable
	1:32,000	D			Uncountable
Quinine bisulphate	1:10,000	S	S	D	Uncountable
	1:16,000	S	D		Uncountable
	1:20,000	D			Uncountable
Quinine hydrobromid	1:10,000	S	S	D	4,000
	1:16,000	D			Uncountable
	1:20,000	D			Uncountable
Liquor thymolis Sputum alone	1:45	S	D		Uncountable
		D†			Uncountable

* S = survived; D = died.
† Type IV pneumococci in peritoneal exudate.

As shown in Table 1, dilutions of ethylhydrocuprein hydrochlorid as high as 1:20,000 had well marked germicidal activity for Type IV pneumococci in the mouth secretions of normal persons; quinin bisulphate and quinin hydrobromid exhibited less activity, but the effects were usually distinct with dilutions reaching 1:10,000. Not infrequently liquor thymolis alone in a 1:4 dilution with the sputum showed feeble pneumococcidal activity; plates showed some germicidal influence on the ordinary bacterial flora of mouth secretions and particularly on pneumococci on the part of the lower dilutions of the various compounds, but these results are to be ascribed mostly to the 1:4 dilution of liquor thymolis alone and particularly in so far as these bacteria other than the pneumococci are concerned.

10 Ibid., 1917, 20, p. 294.

The results of additional experiments conducted after the in vitro-vivo method previously described[10] are shown in Tables 2 and 3.

In these tests thorough mixtures of the mouth secretions of normal persons or the sputum of pneumonia convalescents were made with varying dilutions of cinchonics in a menstruum of liquor thymolis and incubated for varying intervals of time when the pneumococcidal activity was studied by injecting portions intraperitoneally into mice and by plating methods. This technic had

TABLE 2

Germicidal Activity of Varying Dilutions of Ethylhydrocuprein Hydrochlorid in 1:4 Liquor Thymolis Upon Pneumococcic Type IV in the Sputum of Normal Persons *

Sputum C.c.	Cinchonic in 1:2 Liq. Thymolis	Final Dilutions in 1:4 Liq. Thymolis	Animal Tests			48 Hour Blood Agar Plates
			24 Hour	48 Hour	72 Hour	
0.5	0.5 c.c. 1: 2,000	1: 4,000	D†			Sterile
0.5	0.5 c.c. 1: 5,000	1:10,000	S‡	S	S	Sterile
0.5	0.5 c.c. 1: 8,000	1:16,000	S	S	S	Sterile
0.5	0.5 c.c. 1:10,000	1:20,000	S	S	S	Sterile
0.5	0.5 c.c. liq. thymolis alone	1:4 liq. thymolis alone	S	S	S	Few colonies
0.5	0.5 c.c. salt solution	Sputum control	S	S	D§	Uncountable

* Mixtures incubated in thermostat at 35 C. for 10 minutes; 0.5 c.c. injected intraperitoneally and 0.2 c.c. in each plate.
† D = died with convulsions due to toxicity of compound.
‡ S = survived.
§ Control died in 80 hours; Type IV pneumococci in peritoneum and heart.

TABLE 3

Germicidal Activity of Varying Dilutions of Quinin Hydrobromid in 1:4 Liquor Thymolis Upon Pneumococci Type IV in the Sputum of Normal Persons *

Sputum C.c.	Cinchonic in 1:2 Liq. Thymolis	Final Dilutions in 1:4 Liq. Thymolis	Animal Tests			48 Hour Blood Agar Plates
			24 Hour	48 Hour	72 Hour	
0.5	0.5 c.c. 1: 2,000	1: 4,000	D†			Sterile
0.5	0.5 c.c. 1: 5,000	1:10,000	S‡	S	S	Sterile
0.5	0.5 c.c. 1: 8,000	1:16,000	S	S	S	Sterile
0.5	0.5 c.c. 1:10,000	1:20,000	S	S	S	Few colonies
0.5	0.5 c.c. liq. thymolis alone	1:4 liq. thymolis alone	S	S	S	Few colonies
0.5	0.5 c.c. salt solution	Sputum control	S	S	D§	Uncountable

* Mixtures incubated in thermostat at 35 C. for 10 minutes; 0.5 c.c. injected intraperitoneally and 0.2 c.c. in each plate.
† D = died with convulsions due to toxicity of compound.
‡ S = survived.
§ Control died in 80 hours; Type IV pneumococci in peritoneum and heart.

previously yielded us constant and well defined results in a study of the germicidal activity of various cinchonics for pure cultures of various types of pneumococci and in these experiments the results were also of a clear cut character.

Mouth secretions or sputum in amounts of 0.5-1.0 c.c. were mixed in sterile vials with an equal amount of varying dilutions of cinchonics dissolved in diluted liquor thymolis and incubated at 35-37 C. for 10-30 minutes when 0.5 c.c. of each mixture was injected intraperitoneally into white mice and 0.2 c.c. plated; the usual controls of sputum alone and of sputum with liquor thymolis alone were always included.

As shown in Tables 2 and 3, the liquor thymolis alone in dilution of 1 : 4 or 25% protected the mice against the relatively small dose of sputum (0.25 c.c.) of normal persons containing Type IV pneumococci and in conjunction with the two cinchonics employed, namely, ethylhydrocuprein hydrochlorid and quinin hydrobromid, exerted a well marked bactericidal effect as determined by plating the mixtures. The deaths of the mice receiving injections of the 1 : 4,000 dilutions of both compounds were ascribed to the combined toxicity of the drugs and liquor thymolis; the blood of the heart in all such instances was sterile.

The results of a second series of experiments conducted with pneumonic sputum containing virulent Type I pneumococci are shown in Tables 4 and 5. As a 1 : 4, or 25%, solution of liquor thymolis is rather too strong for the purpose of gargling or washing the mouth, these tests were conducted with a 1 : 10 dilution of liquor thymolis in water, the final dilutions after mixing with equal parts of sputum being 1 : 20, or 5%. In these experiments the mixtures of sputum and varying dilutions of cinchonics were incubated in a water bath at 37 C. for one-half hour when 0.5 c.c. of each of the mixtures and controls (equal to 0.25 c.c. undiluted sputum) were injected intraperitoneally into mice and 0.2 c.c. of each plated with blood dextrose agar.

As shown by the results of these experiments the solutions of ethylhydrocuprein hydrochlorid and quinin bisulphate had an appreciable effect in prolonging the lives of many of the mice. In all instances the sputum controls died within 24 hours with Type I pneumococci in the peritoneal cavity and blood of the heart; likewise, the 1 : 20, or 5%, solution of liquor thymolis alone had no appreciable influence in protecting mice against these virulent pneumococci. While a number of the mice injected with the mixtures of cinchonics and sputum died at irregular intervals as so commonly occurs in tests of this kind, the results of numerous experiments indicated that dilutions of ethylhydrocuprein hydrochlorid in sputum as high as 1 : 30,000 and even to 1 : 160,000 had appreciable and frequently well defined pneumococcidal activity, while a 1 : 10,000 dilution almost invariably protected the mice indefinitely. With such cinchonics as quinin bisulphate, dilutions in sputum varying from 1 : 10,000 to 1 : 20,000 were found to possess well defined pneumococcidal activity.

Regarding the bactericidal activity of these dilutions of ethylhydrocuprein hydrochlorid and other cinchonics in 1 : 20 liquor thymolis on the other types of bacteria found in the mouth secretions of normal

and pneumonic persons, our experiments with plating methods were generally negative; as stated and shown in Tables 1, 2, and 3, stronger dilutions of liquor thymolis have an appreciable germicidal activity on these mouth bacteria. Additional experiments have shown that

TABLE 4

Germicidal Activity of Varying Dilutions of Ethylhydrocuprein Hydrochlorid in 1:20 Liquor Thymolis on Pneumonic Type I Sputum *

Sputum C.c.	Cinchonic in 1:10 Liq. Thymolis	Final Dilutions in 1:20 Liq. Thymolis	Animal Tests: Days						Plates
			1	2	3	4	5	6	
0.5	0.5 c.c. 1: 5,000	1: 10,000	S	S	S	S	S	S	Uncountable
0.5	0.5 c.c. 1: 8,000	1: 16,000	S	S	D‡				Uncountable
0.5	0.5 c.c. 1:10,000	1: 20,000	D‡						Uncountable
0.5	0.5 c.c. 1:16,000	1: 32,000	S	S	S	S	S	S	Uncountable
0.5	0.5 c.c. 1:40,000	1: 80,000	S	D‡					Uncountable
0.5	0.5 c.c. 1:80,000	1:160,000	S	S	S	D‡			Uncountable
0.5	0.5 c.c. liquor thymolis 1:10	1:20 liq. thymolis alone	D						Uncountable
0.5	0.5 c.c. salt solution	Sputum control	D†						Uncountable

* Mixtures incubated in water bath at 37 C. for 30 minutes.
† Control; Type I pneumococci in peritoneal cavity and blood of heart.
‡ Type I pneumococci in blood of heart.

TABLE 5

Germicidal Activity of Varying Dilutions of Quinin Bisulphate in 1:20 Liquor Thymolis on Pneumonic Type I Sputum *

Sputum C.c.	Cinchonic in 1:10 Liq. Thymolis	Final Dilutions in 1:20 Liq. Thymolis	Animal Tests: Days						Plates
			1	2	3	4	5	6	
0.5	0.5 c.c. 1: 5,000	1: 10,000	S	S	D‡				Uncountable
0.5	0.5 c.c. 1: 8,000	1: 16,000	S	S	S	D‡			Uncountable
0.5	0.5 c.c. 1:10,000	1: 20,000	S	S	S	S	S	S	Uncountable
0.5	0.5 c.c. 1:16,000	1: 32,000	D‡						Uncountable
0.5	0.5 c.c. 1:40,000	1: 80,000	D‡						Uncountable
0.5	0.5 c.c. 1:80,000	1:160,000	D‡						Uncountable
0.5	0.5 c.c. liquor thymolis 1:10	1:20 liq. thymolis alone	D‡						Uncountable
0.5	0.5 c.c. salt solution	Sputum control	D†						Uncountable

* Mixtures incubated in water bath at 37 C. for 30 minutes; 0.5 c.c. of each injected intraperitoneally into mice.
† Control; Type I pneumococci in peritoneal cavity and heart blood.
‡ Type I pneumococci in heart blood.

dilutions of liquor thymolis with sputum equal to 1 : 2 and 1 : 4 are strongly germicidal for pneumococci and other micro-organisms after an exposure of 10 minutes at 37 C., but dilutions of 1 : 8 and 1 : 10 and higher under the same conditions possess feeble or no appreciable germicidal activity.

After many trials we found that 1 : 10,000 dilutions of ethylhydro-cuprein hydrochlorid or of quinin bisulphate, quinin hydrobromid and other cinchonics in a 1 : 10 dilution of liquor thymolis, constitute mixtures which could be readily used by persons as a mouth wash and gargle. The slightly bitter taste remaining after the use of any of these washes is readily removed by rinsing the mouth with plain water. Further experiments were conducted with two such washes prepared as follows:

Quinin bisulphate .... 0.005 gm.
Liquor thymolis .... 5.0   c.c.
Aqua destillata.............q. s. 50.0   c.c.

Ethylhydrocuprein hydrochlorid... 0.005 gm.
Liquor thymolis .... 5.0   c.c.
Aqua destillata.............q. s. 50.0   c.c.

Mixtures of equal parts of these 1 : 10,000 solutions of quinin bisulphate and ethylhydrocuprein hydrochlorid in 1 : 10 liquor thymolis were made with the sputums of persons convalescent from lobar pneumonia containing virulent Type I pneumococci and also with the mouth secretions of normal persons harboring Type IV pneumococci and incubated in a water bath at 37 C., when 0.5 c.c. of each were injected intraperitoneally in mice at intervals of 1, 2, 3, 5, and 10 minutes to determine the effect on the pneumococci present according to the duration of the lives of the experimental animals. As each mouth wash was diluted with an equal part of sputum in these experiments, the results indicate the influence of 1 : 20,000 dilutions of quinin bisulphate and ethylhydrocuprein hydrochlorid, respectively, in 1 : 20 liquor thymolis, on the pneumococci in both types of sputum.

The results of such experiments with pneumonic convalescent sputum are given in Tables 6 and 7.

With virulent Type I sputums of pneumonia convalescents the mouth wash of quinin bisulphate had but slight effect, prolonging the lives of many mice for but 24-30 hours beyond the duration of life among the controls receiving equal doses of sputum alone (Table 6); the results with ethylhydrocuprein hydrochlorid were usually more marked and particularly with the longer periods of exposure (Table 7).

Similar experiments with the mouth secretions of normal persons harboring virulent Type I pneumococci have not been made, but we

believe that the results would be quite similar to those shown in Tables 6 and 7.

While these experiments have demonstrated the pneumococcidal activity of 1 : 10,000 solutions of ethylhydrocuprein hydrochlorid and quinin bisulphate in 1 : 10 liquor thymolis, a few experiments on the disinfection of the mouths of normal persons harboring Type IV pneumococci have been generally negative. Washing the mouth for

TABLE 6

GERMICIDAL ACTIVITY OF MOUTH WASH OF A 1:20,000 DILUTION OF QUININ BISULPHATE IN 1:20 LIQUOR THYMOLIS ON PNEUMONIC SPUTUM CONTAINING VIRULENT TYPE I ORGANISMS

Exposure at 37 C.	Animal Tests; Days			
	1	2	3	4
1 minute	D*			
2 minutes	S	D*		
3 minutes	S	D*		
5 minutes	D			
10 minutes	S	D*		
Control (1 minute)	D*			
Control (10 minutes)	D*			

* Type I pneumococci in peritoneal cavity and heart.

TABLE 7

GERMICIDAL ACTIVITY OF MOUTH WASH OF A 1:20,000 DILUTION OF ETHYLHYDROCUPREIN HYDROCHLORID IN 1:20 LIQUOR THYMOLIS ON PNEUMONIC SPUTUM CONTAINING VIRULENT TYPE I ORGANISMS

Exposure at 37 C.	Animal Tests; Days			
	1	2	3	4
1 minute	S	D*		
2 minutes	D*			
3 minutes	S	D*		
5 minutes	D			
10 minutes	S	S	S	S
Control (1 minute)	D*			
Control (10 minutes)	D*			

* Type I pneumococci in peritoneum and heart.

3 minutes with a 1 : 20,000 solution of ethylhydrocuprein hydrochlorid in 1 : 10 liquor thymolis or 1 : 10,000 solutions of quinin bisulphate or quinin hydrobromid followed by rinsing the mouth with sterile water and collection of sputum, showed a sufficient number of Type IV pneumococci in 1 c.c. of mouth secretion to kill mice within 24 hours with pneumococci in the blood of the heart. With a wash of 1 : 10,000 ethylhydrocuprein hydrochlorid followed by rinsing the mouth with sterile water and collection of sputum, 0.7 c.c. of sputum at times failed

to kill mice, whereas 0.7 c.c. of sputum collected before using the wash invariably killed within 24 hours with pneumococci in the blood of the heart. Larger doses of sputum collected after using the wash also killed, indicating that the wash had not destroyed all of the pneumococci, but probably had succeeded in reducing their numbers.

Bearing in mind the numerous difficulties in disinfecting the mouth of pneumococci even with powerful and more or less specific anti-pneumococcus agencies as ethylhydrocuprein and other quinin compounds, it is hardly to be expected that complete destruction of all pneumococci in the mouth and upper air passages generally can be accomplished by this means, but for use among physicians, nurses and members of a family in intimate contact with persons suffering with lobar pneumonia the systematic and daily use of washes prepared from 1 : 10,000 solutions of ethylhydrocuprein hydrochlorid or quinin bisulphate in 1 : 10 liquor thymolis, *may serve to destroy virulent pneumococci as they gain access to the mucous membrane of the mouth and upper part of the throat and prevent their proliferation in large numbers;* in this manner and among such groups of persons the systematic use of a mouth wash of this kind held in the mouth and gargled in the throat for at least a minute twice or three times each day may aid in the prophylaxis of lobar pneumonia. Ethylhydrocuprein hydrochlorid by reason of its superior pneumococcidal properties is to be preferred, but owing to the great scarcity of the drug at this time may be substituted by quinin bisulphate; solutions of either stronger than 1 : 10,000 are likely to prove objectionable to most persons. Liquor thymolis in itself appears to aid in the disinfecting process and is well borne in a 1 : 10 dilution serving also to disguise to a large extent the bitter taste of ethylhydrocuprein or other cinchonic.

CONCLUSIONS

Experiments in vitro have shown the high pneumococcidal activity of varying dilutions of ethylhydrocuprein hydrochlorid, quinin bisulphate and other cinchona compounds in liquor thymolis, for Type 1 and Type IV pneumococci in the sputum of convalescents from lobar pneumonia and the mouth secretions of normal persons.

As recent investigations in the epidemiology of lobar pneumonia have indicated that the disease is frequently acquired by transmission of specific virulent types of pneumococci from a recovered person who still harbors in the mouth secretions the organisms responsible for the

disease, or from a healthy carrier of pathogenic pneumococci, it is suggested that the systematic use by the patient and those in close contact with the disease of a mouth wash of 1 : 10,000 solution of ethylhydrocuprein hydrochlorid or quinin bisulphate in 1 : 10 liquor thymolis, may serve to destroy or inhibit the multiplication of the antisepticus, may serve to destroy or inhibit the multiplication of the disease producing pneumococci and thereby aid in the prophylaxis of lobar pneumonia.

COMPLEMENT FIXATION OF STREPTOCOCCI

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Streptococci have been suspected to be the etiologic factor in so many different diseases that for years investigators have tried to determine a satisfactory method by which the various strains could be grouped. Classifications have been made on the basis of (1) morphology, (2) disease source, (3) pathogenicity for animals, (4) power to lake blood, (5) carbohydrate fermentation, (6) immunity tests, especially agglutination and complement fixation, and (7) various combinations of these methods. Comparatively few attempts have been made to classify the streptococcus strains by the complement fixation test. Therefore, it was thought that it might be of value to attempt a classification by the fixation reaction of strains isolated from a number of definite diseases. The following is a brief résumé of the literature on streptococcus complement fixation previously reported.

REVIEW OF LITERATURE

As early as 1904 Besredka[1] inoculated a horse with streptococci, obtaining an immune serum which was specific for that one strain. However, when Besredka and Dopter[2] applied the method to serum from scarlet fever patients, they had no positive results. Schleisser[3] and Sommerfeld[4] also found no positive complement fixation in scarlet fever cases.

Livierato,[5] however, tested the serums of 18 cases of scarlet fever with the following antigens: streptococcus isolated from scarlet fever cases, streptococcus of erysipelas, staphylococcus, diplococci, and typhoid bacillus. All the serums gave positive results with the scarlet fever streptococcus antigen and negative results with the other antigens. Uffenheimer[6] also found that there was specific complement fixation between scarlet fever serum and scarlet fever streptococcus antigen and that the fixation was strongest on the tenth day.

Foix and Mallein[7] obtained specific complement fixation with scarlet fever serum and scarlet fever streptococcus antigen; and in a later series of experiments[8] they observed that serum from 10 of 12 cases of scarlet fever fixed

[1] Ann. de l'Inst. Pasteur, 1904, 18, p. 363.
[2] Ibid., p. 373.
[3] Wien. klin. Wchnschr., 1908, 21, p. 1375.
[4] Arch. f. Kinderh., 1909, 50, p. 38.
[5] Gazz. d. osp., 1907, 28, p. 835.
[6] München. Med. Wchnschr., 1909, 56, p. 2471.
[7] Presse méd., 1907, 15, p. 777.
[8] Presse méd., 1910, 18, p. 215.

9 strains of streptococcus which had been isolated from the throat of scarlet fever patients. Serum of patients with erysipelas and puerperal fever used as control gave negative results. Foix and Mallein concluded that the streptococcus of scarlet fever was a specialized strain.

Castex[9] used serum from persons having erysipelas, scarlet fever, abscess, puerperal fever, pleurisy, and arthritis with streptococcus antigen and obtained positive results with everyone except 3 scarlet fever serums. He considered, therefore, that there were no specific antibodies produced by these various streptococcus strains.

Swift and Thro[10] immunized 5 rabbits with different strains of streptococci, and, while the complement fixation was not absolutely specific, they considered that it might be a means of studying specific streptococcus infections.

Kölmer[11] tested serum from 107 scarlet fever patients, using as antigen a streptococcus isolated from scarlet fever, and secured 11.2% fixations. He also immunized several animals with streptococci from scarlet fever cases and one, as a control, with a streptococcus isolated from a nonscarlatinal tonsillitis. He tested each immune serum against all antigens used in the inoculations and from his results deduced that a strain of streptococci produced a specific antibody up to a certain limit; that is, with low dilutions the specific nature of the antibody was not always observed, but in high dilutions it was.

Major[12] reported a case of endocarditis lenta in which the serum contained antibodies for a Streptococcus viridans which had been previously isolated from the patient. The serum, however, also fixed a hemolytic streptococcus antigen though not a pneumococcus antigen.

Hastings studied the complement fixation of bacteria from arthritis deformans. Twelve of the 24 serums tested gave positive fixation with a Streptococcus viridans antigen. In a later series of experiments[14] he used a polyvalent streptococcus antigen for fixation tests in an effort to detect S. viridans infections. He decided that the serums were so poor in fixing substances and the antigens so poor in fixable substances that the method would not be available for clinical work.

Floyd and Wolbach[15] attempted to correlate streptococcus complement fixations with a classification of streptococcus based on cultural characteristics rather than on the disease from which the strains had been isolated. After performing cross fixation experiments with 7 strains, they concluded that there was a marked degree of specificity between antigens of a given sugar group and their corresponding antiserums.

Kinsella,[16] from the results of fixation tests in a study of subacute streptococcus endocarditis, concluded that the strains causing the disease could be roughly grouped in three classes. In his experiments this classification had no apparent relation to the grouping based on fermentation reactions.

Swift and Kinsella[17] obtained practically the same results in a similar study of the strains isolated in acute rheumatism. Kinsella and Swift[18] then classified nonhemolytic streptococci as a whole, on the basis of complement fixation, and

[9] Presse méd., 1909, 17, p. 324.
[10] Arch. Int. Med., 1911, 7, p. 24.
[11] Arch. Int. Med., 1912, 9, p. 220.
[12] Bull. Johns Hopkins Hosp., 1912, 23, p. 326.
[13] Jour. Am. Med. Assn., 1912, 60, p. 1208.
[14] Jour. Exper. Med., 1914, 20, p. 72.
[15] Jour. Med. Research, 1913, 29, p. 493.
[16] Arch. Int. Med., 1917, 19, p. 367.
[17] Ibid., p. 381.
[18] Jour. Exper. Med., 1917, 25, p. 877.

considered that they could be placed in 3 groups. In looking over their fixation results, they observed as follows: "There was an inverse ratio between the fixing capacity on the part of a serum and the capacity to be fixed on the part of the corresponding antigen." They therefore made 3 groups: a first group in which the antigens used in cross-fixation tests fixed extensively and the corresponding antiserums slightly; a second group in which the serums fixed extensively and the corresponding antigens slightly; and a third group in which there were intermediate reactions. They considered that this classification might be explained by the possible presence of two diverse elements in the group and that some strains entirely consisted of one element, some of the other, and other strains of both.

Aschner[19] by complement fixation tests found that the serums of patients with subacute streptococcus endocarditis give strongly positive fixation with a homologous antigen, a positive with a polyvalent antigen of strains from other endocarditis patients, and a negative with a mixed antigen of similar strains isolated from the throat and local lesions.

TECHNIC

Cultures.—Sixty-five strains of streptococci isolated from definite diseases were used in these tests; 28 were hemolytic and 37 nonhemolytic. Two strains of pneumococci were used as controls. Stock cultures were grown on human blood agar. Cultures for inoculations and antigens were 18-24 hour growths in ascitic dextrose broth.

Antigens.—Bacteria from each culture were removed by centrifugation, washed twice, and suspended in sterile normal salt solution in such quantity that a faintly cloudy, smooth suspension resulted. These suspensions were used for inoculation. For antigens, similar suspensions were heated at 56 C. for 30 minutes.

Immunization.—Twenty-eight rabbits, 3-6 months old, were immunized; 12 against hemolytic strains of streptococcus, 14 against nonhemolytic strains of streptococcus, and 2 against pneumococci. Before immunization the serum of each rabbit was tested with the following antigens: luetic liver extract, ascitic dextrose broth, and the strain selected for inoculation. Only rabbits whose serum gave complete hemolysis with these antigens were used for inoculations. In each rabbit immunization was obtained by intravenous inoculation of the living suspension of the desired strain, at 3 or 4 day intervals, the dosage gradually increasing from 0.25-2 c.c. When 0.02 c.c. of a rabbit's serum gave complete inhibition of hemolysis with $\frac{1}{120}$ of the anticomplementary unit of the corresponding antigen, the rabbit was considered immune. This usually occurred after 7-10 inoculations. Each immune rabbit was bled from the heart. The clear serums were sealed in ampules, inactivated at 56 C. for 30 minutes, and stored in the refrigerator. Immunity was maintained in the rabbits that survived bleeding by weekly subcutaneous inoculation of 2 c.c. of the living bacterial suspension.

Fixation Technic.—The antisheep-rabbit system was used in one-tenth the volume of the classic Wassermann. Each day the anticomplementary unit of antigen was determined, and the hemolytic system was standardized by an amboceptor titration. In the test, the serum remained a constant 0.02 c.c.; the antigen varied from $\frac{1}{4}$-$\frac{1}{120}$ of the anticomplementary unit. Fresh guinea-pig serum 0.1 c.c. of a 1:10 dilution (2 units) was used as complement. Serum, antigen, and complement were incubated at 37 C. for 1 hour. Two units of

[19] Jour. Infect. Dis., 1917, 21, p. 409.

previously titrated antisheep amboceptor and 0.1 c.c. of sheep's corpuscles in 5% suspension were added to each tube, and the whole incubated for 30 minutes. The customary controls (antigen, serum, and hemolytic) were set up with each test.

Each immune serum was tested with each of the streptococcus antigens and with the following control antigens; two pneumococcus, luetic liver, and ascitic dextrose broth as antigen.

COMPLEMENT FIXATION OF STREPTOCOCCUS GROUPED ACCORDING TO HEMOLYSIS OR NONHEMOLYSIS

From the reports on agglutination of streptococcus strains, it seemed probable that the fixation reaction of hemolytic and non-hemolytic strains would be quite different and that a primary division of strains might be made on the basis of hemolysis or nonhemolysis. The hemolytic or nonhemolytic property of a strain was determined by an examination of human blood-agar block plates of each streptococcus. Contrary to expectation, there was marked cross fixation between the two groups. An examination of the results indicated that hemolytic antiserums apparently fixed antigens of hemolytic strains to a greater degree than they did antigens of nonhemolytic strains. The hemolytic antiserums also gave less positive fixation with nonhemolytic antigens than did the nonhemolytic antiserums. It was quite obvious, though, that nonhemolytic antiserums fixed antigens of hemolytic strains at least as well as they did antigens of nonhemolytic streptococci. The percentage of positive fixation of each nonhemolytic serum was then computed with both nonhemolytic and hemolytic antigens (Table 1). The 3 and 4 plus positives were computed together, the 1 and 2 plus positives together, and lastly the total positives (Fig. 1). When these percentages were plotted, it was seen that the contours of the curves of the three were similar. Therefore, the curve of the total fixations only was plotted (Fig. 1). In each case the nonhemolytic antiserum gave a higher percentage of positive fixation with hemolytic antigens than with nonhemolytic antigens, namely, the total fixation with nonhemolytic antigens was 58.2%, while with the hemolytic antigen it was 68.6%.

The percentage of positive fixation of hemolytic immune serums with hemolytic and nonhemolytic antigens was computed in the same way (Table 1). There was the same relation between the curves of the total positives, strongly positives, and faintly positives as was noted with the nonhemolytic antiserums (Fig. 2). Here, however, the percentage of positive fixation was slightly greater with the homologous

group antigens than with the heterologous group antigens, namely, the total positive fixation with hemolytic antigens was 61.9% and with non-hemolytic 48.4%. From these results it appeared that the nonhemolytic antiserums were less specific in their action than the hemolytic.

TABLE 1

PERCENTAGE OF FIXATION OF ANTISERUM WITH HEMOLYTIC AND NONHEMOLYTIC ANTIGENS

Antiserums	Hemolytic Antigens		Nonhemolytic Antigens	
	Number of Tests	Percentage of Fixation	Number of Tests	Percentage of Fixation
Hemolytic				
A6..........................	11	54	16	62
A8..........................	28	71	37	62
A10.........................	28	53	37	46
D5..........................	28	60	36	33
D7..........................	28	71	36	47
E1..........................	28	75	37	62
E2..........................	28	18	37	16
E3..........................	28	75	36	41
L1..........................	2	50	5	83
L4..........................	17	41	26	42
M1..........................	28	79	37	65
S1..........................	22	81	33	70
Total.....................	276	62	373	48
Nonhemolytic				
A1..........................	15	40	24	50
A2..........................	28	82	37	78
A7-1........................	14	78	24	66
A7-2........................	28	78	37	76
A11.........................	23	65	32	34
B1..........................	28	85	36	69
B2-1........................	10	30	12	33
B2-2........................	28	67	36	74
G1..........................	28	78	36	61
G2..........................	28	78	36	33
L13.........................	28	53	36	44
L14.........................	28	96	36	86
M14.........................	28	39	37	48
T1..........................	27	58	36	38
Total.....................	341	68	455	58

COMPLEMENT FIXATION OF STREPTOCOCCI GROUPED ACCORDING TO DISEASE

Antigens were made of each of the 65 strains and an effort was made to immunize at least 2 rabbits in each disease group.

GROUP A (*Acute Poliomyelitis*).—Eleven antigens and 8 antiserums were employed in this group. The results of the cross complement fixation tests within the group and with several control antigens has been previously reported.[20] At that stage in the tests there appeared to be almost complete group specificity. Since that report, though, the immune serums have been tested with the antigens of the other disease

[20] Mathers and Howell: Jour. Infect. Dis., 1917, 21, 292.
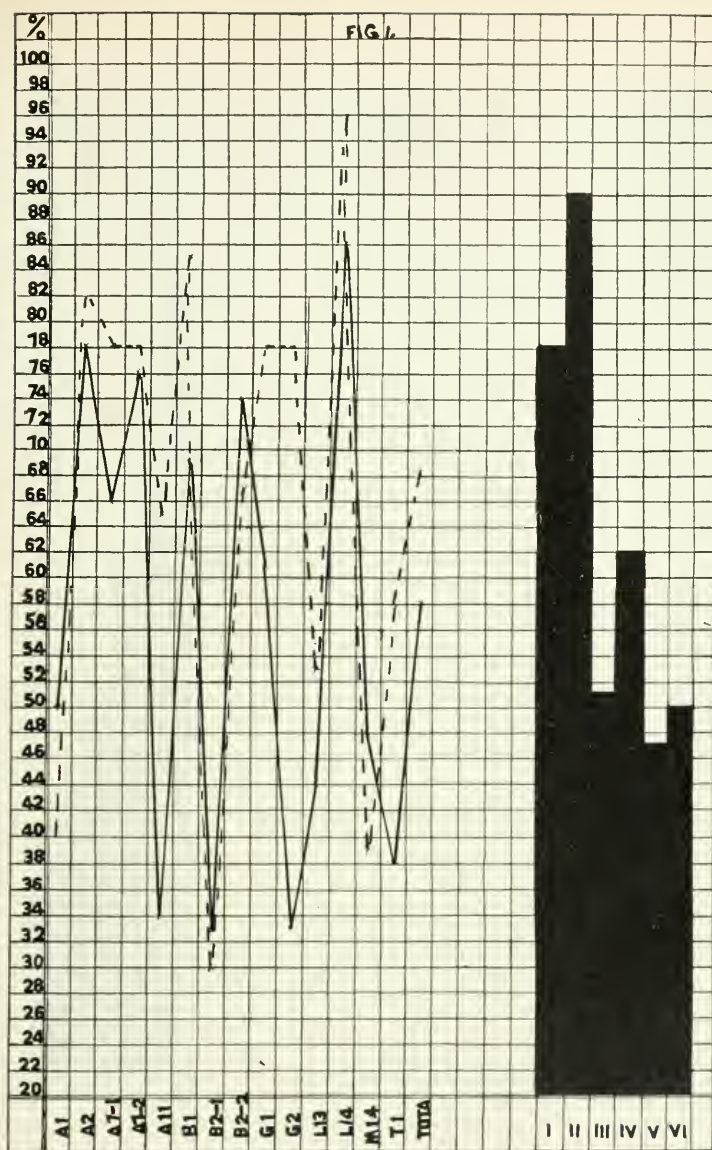
Fig. 1.—Solid line = percentage of fixation of nonhemolytic serums with nonhemolytic antigens. Broken line = percentage of fixation of nonhemolytic serums with hemolytic antigens.

I 455 nonhemolytic antigens, 265 total fixations = 58%
II 341 hemolytic antigens, 234 total fixations = 68%
III 455 nonhemolytic antigens, 142 fixations (1+, 2+) = 31 %
IV 341 hemolytic antigens, 131 fixations (1+, 2+) = 38%
V 455 nonhemolytic antigens, 123 fixations (3+, 4+) = 27%
VI 341 hemolytic antigens, 103 fixations (3+, 4+) = 30%
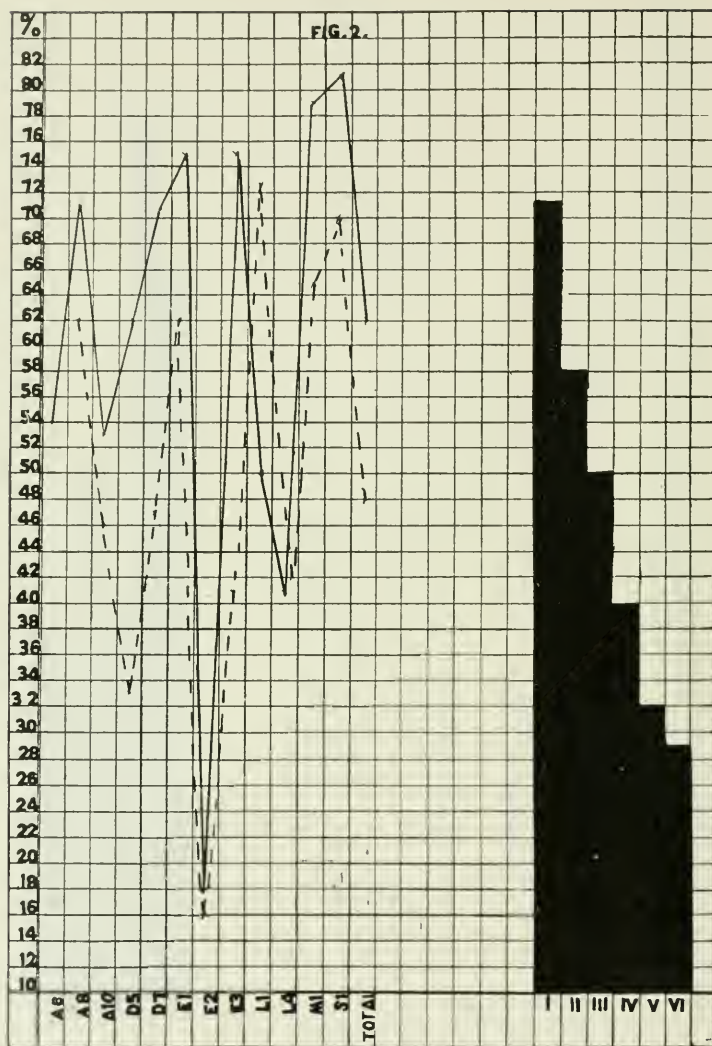
Fig. 2.—Solid line = percentage of fixation of hemolytic antiserums with hemolytic antigens. Broken line = percentage of fixation of hemolytic antiserums with nonhemolytic antigens.

    I 277 hemolytic antigens, 172 total fixations = 61%
   II 373 nonhemolytic antigens, 181 total fixations = 48%
 III 277 hemolytic antigens, 110 fixations (1+, 2+) = 40%
 IV 373 nonhemolytic antigens, 111 fixations (1+, 2+) = 30%
  V 277 hemolytic antigens, 62 fixations (3+, 4+) = 22%
 VI 373 nonhemolytic antigens, 70 fixations (3+, 4+) = 19%

groups with the result that they gave considerable scattered fixation. Two rabbits in this group were immunized against the same strain (A7), and it was interesting to note that the antiserums (A7-1 and A7-2) did not give parallel complement fixation reactions either in the homologous or heterologous groups.

Group B (*Endocarditis*).—Six strains were isolated from the blood of patients with endocarditis. Two rabbits were immunized against strains B1 and B2, respectively. Rabbit B2 died after immunization and another rabbit was inoculated with the B2 strain. The complement reactions of the antiserums B2-1 and B2-2 were fairly similar. The 3 immune serums gave strong fixation with the endocarditis antigens, but fixed almost equally well the antigens of the sepsis and measles groups. There was also considerable cross fixation with the other streptococcus group antigens. The antiserums gave fixation, ranging from + to ++++ with the 2 pneumococci antigens, and B2-2 gave a weakly positive reaction with luetic liver antigen. The serum would seem, therefore, to give nonspecific reactions.

Group D (*Sepsis*).—The strains used in this group were isolated from the blood of patients with severe sepsis: puerperal sepsis (D1, D2, D7); secondary to empyema (D4, D6); post-operative (D3); secondary to brain abscess (D5); following acute mastoiditis (D8); from meningitis (D9). Strains D5 and D7 were used for immunization. There was no apparent group fixation. Antigens from the same disease did not have similar fixative power with the various antiserums.

Group E (*Erysipelas*).—The three strains were isolated from erysipelas, E1 from a bleb and E2 and E3 from blood cultures. Antigens and immune serums of each strain were used in the fixation tests. Considering this group alone, the group fixation was apparently specific, but examining the chart as a whole, the usual cross fixations with other group antigens were observed.

Group G (*Acute Articular Rheumatism*).—The two strains were isolated from the blood of patients with acute articular rheumatism. These strains were obtained from Swift and Kinsella.[17] The results of the group fixation corresponded to those obtained by them in that the antiserum G1 fixed with antigen G1, but not with antigen G2 and vice versa. There was considerable cross fixation with the other streptococcus groups. The cross fixation with the endocarditis group composed of nonhemolytic strains that were similar culturally, was very slight, while that with the sepsis group composed mostly of hemolytic

strains, was especially strong. The fixation with the pneumococcus antigen was strongly positive.

GROUP L (*Upper Respiratory Tract Infections*).—This rather general group included strains isolated from the nasal discharge in acute and chronic coryza (L1-L5), from sputum in la grippe (L5-L8), and from the ulcers in tonsillitis (L8-L14). The tonsillitis antiserums (L13, L14) fixed the majority of the tonsillitis antigens; with that exception, there was no group specificity.

GROUP M (*Measles*). — In this group, 13 strains were used as antigens. Eleven strains were isolated from measles cases and 2 (M10, M13) from German measles. Two antiserums (M1, M14) were used in the test. M1, a hemolytic strain, was isolated from the throat of a measles patient, and M14, a nonhemolytic strain, from the blood of a measles patient just before the appearance of the rash. The fixation of the 2 immune serums were quite different. M1 fixed all the acute poliomyelitis antigens while M14 gave only a slight fixation with 2 antigens in that group. Both antiserums fixed a number of antigens in the measles and upper respiratory tract groups, but frequently not the same antigen. There was fixation with the pneumococcus antigens, slight with M1, strong with M14.

GROUP S (*Scarlet Fever*). — There was only one scarlet fever antigen and antiserum. The strain was isolated from the blood of a scarlet fever patient. It was a very virulent organism and 2 rabbits died in the course of immunization. The third rabbit did not develop an immune serum of high titer for the strain. The fixation with antigens of the sepsis group was more strongly positive than with the homologous antigen. There was cross fixation with other group antigens, especially with endocarditis and pneumococcus strains.

GROUP T (*Thrombosis*).—This was a nonhemolytic streptococcus from the blood of a patient with thrombosis. Only one rabbit was immunized before the strain was lost.

GROUP X (*Miscellaneous*). — This group contained miscellaneous strains: 2 from lung abscesses, 1 from a tooth abscess, 1 from the blood of the umbilical cord, and 1 from spinal fluid in meningitis. They were used as antigens only.

GROUP P (*Pneumonia*). — Two rabbits were immunized with 2 strains of pneumococcus as a control group. Unfortunately, both antiserums were probably nonspecific since they had a high percentage of

TABLE 2

Complement Fixation Tests

Rabbit Anti-serums	Streptococcus Antigens																																																												Lactic Liver Extract	Ascitic Dextrose Broth
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	B1	B2	B3	B4	B5	B6	D1	D2	D3	D4	D5	D6	D7	D8	D9	E1	E2	E3	G1	G2	L1	L2	L3	L4	L6	L7	L8	L9	L10	L11	L12	L13	L14	M1	M2	M4	M5	M6	M7	M8	M9	M11	M12	M14	M10	M13	S1	T1	X1	X2	X3	X4	X5	P1	P2		
A1	4	3	0	3	4	2	2	3	4	4	4		0					2	2	2	0	3	2	0	3	3	0	0	0	0	0		0		0						0	1			0	0	0	0		0	0	1	1	0		2	2	3	3	2	0	0	0	0
A2	3	4	4	3	4	3	3	4	4	4	3	0	2	4	4	2	2	2	2	2	0	3	2	0	3	3	3	2	2	4	4	0	0	4	3	2	2	2	4	4	0	4	3	4	4	0	0	0	0	1	2	0	1	0	0	0	1	2	2	2	2	1	3	0	0
A6	1	4	0	2	3	4	4	4	2	4	0	1	1	0								2	4	1	0	8	3	2	0	3	3	1	0		0				3	0					0	0	0	0	0	4	0	0	0	0	0	0	1		0	2	1	3	0	0	0
A7-1	0	3	1	3	1	1	4	1	1	2	3	3	2	2	4	0	2								2	4	3	2	0	3	3	0	1	3				3	0					0	0	0	0	4							1	4		0					0	0	0
A7-2	3	2	3	2	3	4	4	0	1	3	1	1	3	1	0	1	1	3	3	2	0	3	3	2	0	2	3	1	1	2	2	4	2	1	0	0	1	2	4	3	0	4	1	0	1	1	0	4	0	0	0	1	2	0	2	1	2	3	2	4	3	2	0	1	0
A8	1	4	0	3	3	3	1	4	4	4	0	2	1	3	4	0	0	0	3	1	0	3	3	2	0	2	4	1	0	0	3	0	2	1	2	3	0	2	3	3	0	4	0	4	0	4	4	2	0	0	0	3	2	0	0	2	3	1	4	1	1	2	0	0	0
A10	3	4	2	2	0	1	4	4	2	4	0	1	1	0	1	0	0	0	2	0	1	2	0	0	0	1	0	0	0	3	3	0	2	0	0	1	0	1	0	0	0	3	2	2	1	0	0	0	0	0	2	1	0	0	0	3	0	1	0	0	4	1	2	0	0
A11	4	4	0	2	4	2	4	4	4	2	4	2	1	0	0	0	0	2	8	0	2	0	2	0	2	0	2	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	3	0	0	0	3	0	0	0	0	0		2	0	0	0	0	0	0
B1	2	4	1	3	2	1	0	2	2	3	2	4	4	0	4	4	4	3	2	3	3	3	2	3	3	4	1	1	0	1	0	3	0	3	4	0	2	0	2	2	2	0	3	3	1	0	0	1	2	2	0	3	0	0	2	2	2	0		1	1	2	2	1	4
B2-1	0	0	0	0	0	0	0	0	0	0	0	4	2	2	0	0	0										0	1	0			0		0											1											1	0	0							0
B2-2	4	4	1	2	0	1	0	0	0	2	1	2	4	2	3	4	4	2	3	2	4	1	3	2	3	4	0	1	3	4	0	1	0	2	2	2	1	4	2	4	3	2	0	0	1	0	2	0	4	3	0	1	2	3		1	4	0	1	2	2	3	1
D5	0	2	0	0	0	1	2	0	0	0	2	0	0	0	0	1	0	0	1	0	1	4	1	0	2	1	2	0	1	0	0	1	1	1	0	2	0	3	0	0	1	0	0	4	2	0	0	1	0	1	0	0	0	1	1	0	0	2	0	3	1	2	2	0
D7	0	0	0	0	0	1	0	0	1	0	2	0	0	0	0	0	0	1	4	1	2	4	3	4	2	0	3	2	1	2	0	0	2	2	3	2	0	2	3	4	2	1	1	4	2	0	3	0	0	2	1	1	2	3	3	0	3	0	3	0	3	1	0	0
E1	1	0	1	1	3	0	0	2	2	0	2	1	1	4	0	0	0	2	2	1	2	4	4	4	0	0	3	2	4	2	2	1	3	1	2	0	2	1	2	0	4	2	1	2	1	2	2	0	0	2	1	4	2	1	2	2	0	0	1
E2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3	3	2	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	4	1	0	1	0	1	0	0
E3	0	0	0	1	0	1	0	0	2	1	1	0	0	0	0	0	0	3	4	1	3	3	4	0	0	0	2	2	4	0	0	0	0	1	0	0	0	0	1	0	2	2	4	4	3	1	0	0	0	0	1	2	1	2	2	4	3	0	3	1
G1	0	1	0	2	0	1	0	0	4	3	3	2	2	0	1	2	0	2	2	0	3	4	3	3	2	1	1	1	2	4	0	1	0	1	0	0	2	2	2	2	2	2	4	3	0	0	0	0	2	0	0	0	0	0	3	4	4	3	0	3	4	4	0	0
G2	0	2	0	3	0	0	0	0	2	2	0	0	0	0	0	0	0	1	4	2	3	2	3	3	1	1	0	1	3	0	3	1	0	2	3	2	0	2	2	0	2	4	0	3	1	1	0	0	1	1	2	0	2	0	0	0	0	2	3	0	3	4	3	0	3	2
L1	1	1	1		0							3	1	0													4					4																								3		0	1	0	0	0	0	2	0	0
L4	0	0	0	0	0	0	0	0	0	0	2	3	1	0	0	0	0	0	1	3	3					3	4	1	0			0	0	4	2	2	0	0	0	0	2	0			0	0	2	0	0						1	0	4	0	0	3	0	1	0	0
L13	0	1	0	2	0	1	0	0	0	0	2	1	0	0	0	1	0	0	1	3	3	4	1	2	1	0	1	1	2	0	0	0	4	0	0	0	0	0	1	0	2	4	4	3	0	0	0	0	0	0	0	2	0	2	0	4	0	0	3	0	4	3	0	0
L14	0	4	3	3	2	1	1	2	3	3	2	4	3	4	4	4	4	2	3	3	3	3	4	2	2	3	4	4	4	4	4	1	2	1	4	2	3	2	2	2	4	4	4	4	4	1	2	4	1	0	3	4	4	3	2	2	1	0	2	3	0	3	2	2
M1	3	3	4	4	3	3	4	2	4	4	2	2	2	0	0	0	0	3	2	1	2	0	3	1	2	1	0	0	2	0	0	1	0	4	2	3	2	3	4	0	2	1	2	0	4	0	0	0	0	0	3	3	3	3	4	1	1	4	2	1	0	2	0	1	2
M14	0	0	0	0	0	1	0	1	0	0	2	0	0	0	1	1	0	2	2	0	0	0	0	0	0	0	0	0	0	1	1	0	0	4	3	0	2	0	3	0	1	1	1	0	1	0	2	0	0	2	3	1	2	0	4	0	3	2	0	0	1	0	0	0	3	4
S1	1	1	0	2	1	0	2	2	0	2	3	1	1	4	4	4	3	4	4	3	3, 3	0	1	2	1	4	1	2	1	4	1	4	2	2	0	0	4	1	0	2	0	0	4	1	0	2	0	0	4	0	0	0	3	3	1	0	4	3	3	0
T1	1	1	0	2	1	0	0	0	2	0	0	1	0	0	0	0	3	0	1	2	0	0	4	3	0	1	1	1	0	4	1	3	0	3	0	0	0	0	2	3	0	0	1	0	1	4	1	8	2	1	0	4
P1	3	2	3	3	4	4	4	3	3	4	0	1	1	4	3	0	2	1	1	0	2	2	0	1	2	3	1	0	0	0	3	1	0	4	2	2	0	1	1	3	0	0	0	3	0	2	0	0	2	0	0	0	2	4	4	4	0	4	1	0	4	2	2	2	3	4	2	2
P2	2	2	0	0	0	2	2	2	2	2	4	3	0	0	3	0	0	0	4	4	3	3	2	4	4	4	1	0	0	0	1	3	0	3	3	4	3	2	4	4	0	0	4	2	0	0	0	0	3	0	0	0	1	3	3	0	0	2	0	3	2	2	4	0	2	1
Normal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = complete hemolysis.
1 = inhibition of hemolysis in 1/4-1/8 of anticomplementary unit.
2 = inhibition of hemolysis in 1/16 of anticomplementary unit.
8 = inhibition of hemolysis in 1/32-1/64 of anticomplementary unit.
4 = inhibition of hemolysis in 1/128, etc., of anticomplementary unit.

fixation with practically all groups and also gave a weak fixation with luetic liver antigen.

A normal rabbit serum did not inhibit hemolysis with any antigen. The results of the fixation test appear in Table 2.

The percentage of positive fixation of the serums in each group was computed for the homologous group antigen and for the heterologous group antigens (Table 3) and the percentages plotted (Fig. 3). In
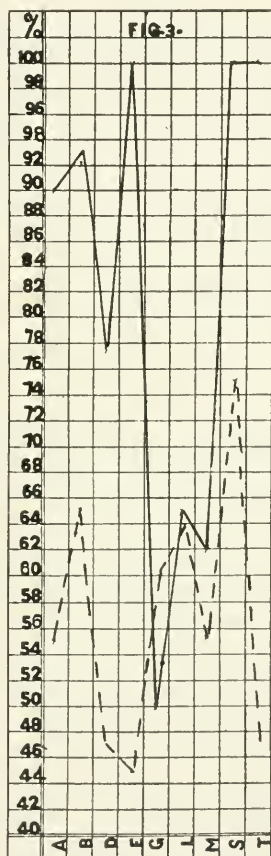
Fig. 3.—Solid line = percentage of fixation of disease group antiserums with homologous antigens. Broken line = percentage of fixation of disease group antiserums with heterologous antigens.

A = Anterior poliomyelitis strains.
B = Endocarditis strains.
D = Sepsis strains.
E = Erysipelas strains.
G = Arthritis strains.

L = Upper respiratory tract infection strains.
M = Measles strains.
S = Scarlet fever strains.
T = Thrombosis strains.

every group except one (Group G) the percentage of positive fixation was greater with the homologous antigens than with the heterologous antigens, the more definite disease groups showing a greater difference between the 2 percentages.

There are too few immune serums and antigens in each group to justify drawing any definite conclusions, but the data suggest that there is no clear cut group specificity.

TABLE 3

PERCENTAGE OF FIXATION OF DISEASE GROUP ANTISERUMS WITH HOMOLOGOUS AND HETEROLOGOUS GROUP ANTIGENS

Antiserums			Homologous Antigens		Heterologous Antigens	
Number	Group		Number of Tests	Percentage of Fixation	Number of Tests	Percentage of Fixation
8	A	(Acute poliomyelitis)...................	88	90	331	55.5
3	B	(Endocarditis)...........................	15	92·	134	64.9
2	D	(Sepsis.................................	18	77	110	47.2
3	E	(Erysipelas)............................	9	100	185	44.8
2	G	(Acute rheumatic fever)...............	4	50	122	60
4	L	(Upper respiratory tract infections)...	40	65	136	64
2	M	(Measles)...............................	26	62.5	104	55.7
1	S	(Scarlet fever).........................	1	100	55	75
1	T	(Thrombosis)...........................	1	100	62	47.3

COMPLEMENT FIXATION OF STREPTOCOCCI GROUPED ACCORDING TO CARBOHYDRATE FERMENTATION

The mediums used for the fermentation tests were sugar-free broth (peptone 1%, salt 0.5%, Liebig's beef extract 0.25%) plus 1% of the various carbohydrates. The reaction was titrated to plus 1.0 with phenolphthalein as an indicator. The following carbohydrates were used: dextrose, lactose, saccharose, maltose, salicin, inulin, raffinose, and mannit. All cultures were inoculated into one kind of sugar broth medium on the same day. Also control tubes of the same medium were used. The cultures were incubated at 37.5 C. for 8 days, and the readings were made at the end of that time. Five c.c. of the broth in each tube was thoroughly mixed in a flask with 45 c.c. of freshly boiled distilled water and titrated in the cold against N/20 NaOH, using phenolphthalein as an indicator. The average titration of the 2 control tubes was noted, and, arbitrarily, everything above this point was considered positive fermentation.

The streptococci were then grouped according to both Holman's[21] and Andrewes and Horder's[22] classifications. However, so many of

[21] Jour. Med. Research, 1916, 34, p. 377.
[22] Lancet, 1906, 2, pp. 708, 775, 852.

TABLE 4

STREPTOCOCCUS STRAINS GROUPED ACCORDING TO CARBOHYDRATE CLASSIFICATION

Group	Strains
Pyogenes...............	A10, D6, D7, D8, D9, E1, E2, L4, L7, L8, L10, L12, M1, M7, M10, S1, X2
Mitis...................	A2, A4, A5, B2, B3, B4, B5, B6, D1, G1, L2, L3, L6, M4, M5, M11, M13
Fecalis.................	A1, B1, G2, L9, L13, L14, M6, X4, X5
Infrequens.............	A6, A8, A9, D3, D4, D5, L1, L5, M8
Ignavus................	A3, A7, A11, M12
Salivarius..............	M9, M14, T1, X1
Anginosus.............	D2, X3
Hemolyticus I.........	E3
Equinus................	M2

TABLE 5

PERCENTAGE OF FIXATION OF ANTISERUMS AND ANTIGENS GROUPED ACCORDING TO CARBOHYDRATE CLASSIFICATION

Antiserums		S. Pyogenes		S. Mitis		S. Fecalis		S. Infrequens	
No.	Group	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive
7	Pyogenes..........	111	60	114	47	70	57	57	60
4	Mitis..............	48	80	56	74	33	72	30	53
5	Fecalis.............	78	70	76	60	48	56	40	77
4	Infrequens........	41	61	51	57	25	65	23	65
3	Ignavus...........	41	70	45	66	24	46	19	63
2	Salivarius.........	34	56	34	44	20	40	17	35
1	Hemolyticus.......	17	71	17	30	10	25	9	77

the strains were variants of the main groups of Andrewes and Horder that their classification was discarded and Holman's alone used. The majority of the strains were in the following groups: S. pyogenes. S. mitis and S. fecalis (Table 4).

The immune serums and the antigens were then grouped according to this sugar classification, and the complement fixation reactions of each serum and antigen noted. The percentage of positive complement fixation of the immune serums of each sugar group, with the homologous and heterologous sugar group antigen, was computed. The results appear in Table 5. Although there are too few strains in some groups to draw definite conclusions, taken as a whole the table indicates that there is little or no sugar group specificity. It is interesting to note that group S. pyogenes shows a slight degree of specificity, while S. mitis and S. fecalis antiserums have a higher percentage of fixation with heterologous antigens than with their homologous group antigens — a result similar to that given by the hemolytic and non-hemolytic antiserums in the classification based on hemolysis and non-hemolysis. The results differ markedly from that of Floyd and

Wolbach,[15] who considered that complement fixation tests while not absolutely specific indicated a marked degree of specificity between strains of a given sugar group and their corresponding antiserums.

COMPLEMENT FIXATION OF STREPTOCOCCI GROUPED ACCORDING TO OTHER METHODS

The percentage of fixative power of each immune serum and the percentage of fixative power of its corresponding antigen was computed (Table 6) and a curve plotted (Fig. 4) in order to see whether

TABLE 5

PERCENTAGE OF FIXATION OF ANTISERUMS AND ANTIGENS GROUPED ACCORDING TO CARBOHYDRATE CLASSIFICATION

S. Ignavus		S. Salivarius		S. Anginosus		S. Hemolyticus I		S. Equinus	
No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive	No. Tests	Per Cent. Positive
28	52	23	56	12	64	7	71	7	17
15	47	11	86	6	83	4	50	3	33
20	45	15	60	9	66	5	60	5	20
12	58	8	37	5	80	3	33	3	0
11	73	8	62	4	75	3	33	3	33
8	33	7	57	4	33	2	0	2	0
4	50	3	100	2	100	1	100	1	0

TABLE 6

PERCENTAGE OF FIXATIVE POWER OF ANTISERUMS AND ANTIGENS

Strain	Antiserum Fixation		Antigen Fixation	
	Number of Times Used	Percentage of Fixation	Number of Times Used	Percentage of Fixation
A1	41	38	26	58
A2	65	80	26	77
A6	27	61	26	77
A7-1	38	71	25	42
A7-2	65	77	25	42
A8	65	66	26	54
A10	65	49	26	61
A11	55	40	26	69
B1	64	78	26	68
B2-1	22	32	26	65
B2-2	64	72	26	65
D5	64	45	20	55
D7	64	56	20	55
E1	65	69	26	68
E2	65	17	26	72
E3	64	56	26	52
G1	64	69	26	50
G2	64	60	26	48
L1	8	86	19	63
L4	43	58	26	56
L13	64	48	18	100
L14	64	91	19	54
M1	65	71	26	64
M14	65	51	20	50
S1	36	75	26	76
T1	63	48	16	62
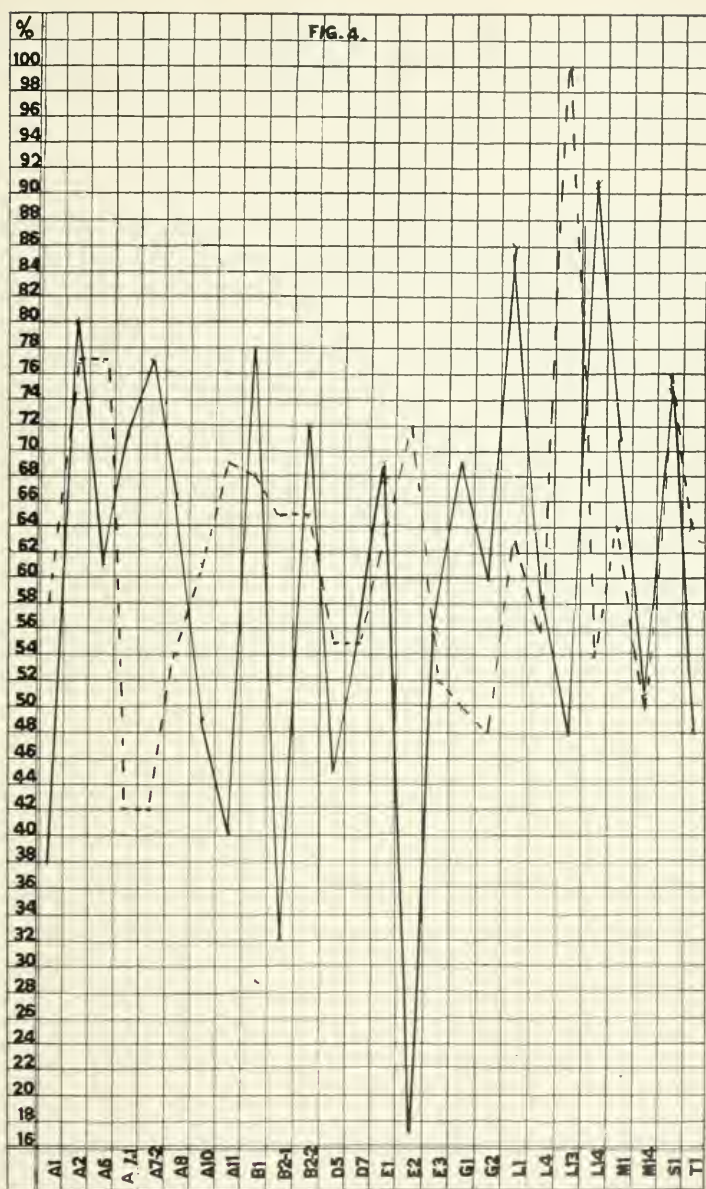
Fig. 4.—Solid line = percentage of fixative power of antiserums. Broken line = percentage of fixative power of antigens.

there was an inverse ratio between the two as suggested by Kinsella and Swift.[18] This inverse ratio was observed in only 38.4% of the immune serums and homologous antigens.

No other grouping of the positive fixations seemed possible from an examination of the whole table.

CONCLUSIONS

No correlation was observed between complement fixation tests and groups based on hemolysis and nonhemolysis. Nonhemolytic antiserums apparently gave even less specific complement fixation reactions than hemolytic antiserums.

There was, on the whole, only slight correlation between complement fixation tests and groups based on disease. The more definite disease groups, however, such as anterior poliomyelitis, erysipelas, and endocarditis tended toward group specificity.

There was no obvious relation between complement fixation tests and groups based on the fermentation of carbohydrates.

No inverse ratio was observed between the fixative power of an antigen and its antiserum.

The positive fixations could not be grouped in any way that would justify a classification of streptococci based on the complement fixation test.

A NEW MICROSCOPIC METHOD OF COUNTING BACTERIA ADAPTABLE TO ALL GRADES OF RAW AND PASTEURIZED MILK

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One of the greatest difficulties in the way of the bacteriologic control of market milk is the lack of a suitable means of determining the number of bacteria per c.c. at all stages of production and handling. The plate method is not adaptable, as it requires a laboratory and at least a few days for incubation of the plates, during which time the milk is consumed before the count is known. The microscopic method of counting bacteria in which the $\frac{1}{100}$ c.c. pipet is used has little accuracy until the milk contains about 300,000 bacteria per c.c.

It is with the above facts in view that the writer has been carrying on during several years a somewhat continuous attempt to find a method of counting bacteria in milk when the number per c.c. ranges from 3000 to 300,000. The following method has been found successful in so far as it has been used as is shown by the results here reported. However it is realized that a final verdict can be given only after several laboratories report favorable results.

THE METHOD

The method is based on the fact that a water suspension of aluminum hydroxid readily collects the bacteria in milk and that the centrifuge is able to throw down the aluminum hydroxid containing the bacteria to one end of the centrifuge tube leaving the fat, casein, and water very largely behind. The aluminum hydroxid is readily transferred to a glass slide and dried down. As aluminum hydroxid shrinks to a very considerable degree on drying a thin microscopic film is made possible. In staining the films it is found that the hydroxid has slight affinity for methylene blue and that on washing them with water a clear field is obtained in which the bacteria stand out by contrast.

THE METHOD IN DETAIL

Preparation of the Hydroxid Suspension.—Mix equal parts of N/20 $AlK(SO_4)_2.12H_2O$ and N/20 NaOH. After precipitation is complete wash the precipitate thoroughly by decantation using scrupu-

lously pure distilled water, keeping in mind that any dirt in the wash water will be taken up by the aluminum hydroxid and will appear in the film under the microscope. Make up to original volume.

Procedure.—1. Add to the sample of milk to be analyzed enough of the washed aluminum hydroxid precipitate suspension so that it becomes 20% of the mixture. Shake thoroughly for several minutes.

2. Add 2.5 c.c. of the above mixture to a centrifuge tube holding this amount when stoppered. (Use plain centrifuge tube open at both ends for stoppers.)

3. Centrifuge for 15 minutes at 5000 revolutions per minute.

4. Pull stopper at cream end of tube and remove the cream with a needle allowing the milk to run out. Then carefully pull the stopper at the other end of the tube and transfer the plug of aluminum hydroxid to a clean glass slide laying over glass or cardboard ruled off
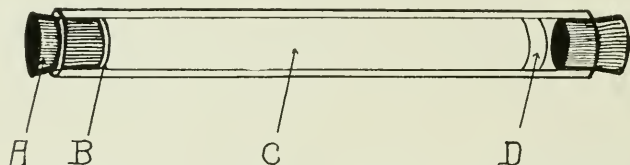
Fig. 1.—Centrifuge tube. Length of the tube is 8 cm.; inside diameter 8 mm. *A*, rubber stopper; *B*, plug of $Al_2(OH)_6$; *C*, milk free from fat and $Al_2(OH)_6$; *D*, butter fat.

in sq. cm. By use of a needle or loop grind up the precipitate on the slide and spread it evenly over an area of 2 sq. cm. using a loopful of sterile water if necessary for uniform spreading.

5. Place in incubator until nearly dry, then finish drying at room temperature.

6. Heat slightly several inches above Bunsen flame.

7. Place in tray of toluene and agitate tray several minutes to remove butter fat, than take slides out of tray and allow the toluene to evaporate from them.

8. Place slides in 95% alcohol to remove all traces of toluene.

9. Stain in 25% saturated aqueous solution of methylene blue until the film reaches a proper depth.

10. Dry in the warm air above a Bunsen flame and examine under the microscope, using an oil immersion lens of which the number of fields per sq. cm. has been determined.

TABLE 1

Comparison of the Hydroxid Method of Counting Bacteria in Milk with the Plate Method and the Breed Method

Sample of Milk, Number	Kind of Milk	Plate Method Count	Hydroxid Method Count		Breed Method Count	
		Bacteria per C.c.	Bacteria per Average Field	Bacteria per C.c.	Bacteria per Average Field	Bacteria per C.c.
101	Raw	92,500	35.2	105,600	0.5	150,000
102	Raw	114,000	52.0	156,000		
103	Raw	103,600	60.9	182,700	0.2	100,000
104	Raw	326,600	119.4	358,200	2.3	690,000
105	Raw	68,000	25.7	77,000	0.4	120,000
106	Raw	353,300	141.5	424,500	1.5	450,000
107	Raw	263,300	123.6	370,800		
108	Raw	170,000	108.6	325,800	1.0	300,000
109	Raw	88,000	61.8	155,400	0.5	150,000
110	Raw	108,000	55.3	165,900	0.9	270,000
111	Raw	82,000	40.8	122,000		
112	Raw	16,600	7.6	22,800		
113	Raw	87,000	47.2	141,600	0.7	210,000
114	Pasteurized	8,060	5.5	16,500		
115	Pasteurized	6,630	3.1	9,300		
116	Raw	106,000	36.4	104,200	0.7	210,000
117	Raw	294,600	143.2	429,600	1.2	460,000
118	Raw	230,000	99.6	298,800	1.6	480,000
119	Raw	73,300	23.3	69,900	0.1	30,000
120	Raw	142,300	74.5	223,500	1.7	510,000
121	Raw	266,300	156.4	469,200	2.1	630,000
122	Raw	126,000	63.5	180,500	0.6	180,000
123	Raw	311,600	118.0	354,000	0.7	210,000
124	Raw	666,000	267.7	803,100	1.4	420,000
125	Raw	5,430,000	Thousands	....	27.4	8,220,000
126	Raw	4,160,000	Thousands	....	29.2	8,760,000
127	Raw	466,000	205.9	617,700	2.8	840,000
128	Raw	936,000	296.1	888,300	3.7	1,110,000
129	Raw	3,200,000	Thousands	....	19.0	5,715,000
130	Raw	30,600	14.9	44,700	0.2	60,000
131	Raw	3,083	1.8	5,400		
132	Raw	930,000	307.6	922,800	3.5	1,065,000
133	Raw	1,700,000	720.0	2,157,000	7.6	2,295,000
134	Raw	923,300	470.0	1,410,600	4.5	1,365,000
135	Raw	676,000	365.0	1,095,000	5.0	1,500,000
136	Raw	846,000	319.4	958,200	3.6	1,080,000
137	Raw	1,253,000	570.0	1,710,000	7.1	2,130,000
138	Raw	690,000	367.6	1,102,800	5.2	1,560,000
139	Raw	44,600	21.7	65,100	1.3	390,000
140	Raw	135,300	71.4	214,200	1.1	330,000
141	Raw	1,466,000	Thousands	....	30.2	9,060,000
142	Raw	11,033,000	Thousands	....	72.8	21,840,000
143	Raw	18,930	7.9	23,700		
144	Raw	1,100,000	517.6	1,552,000	6.0	1,600,000
145	Pasteurized	13,500	4.5	13,500		
146	Pasteurized	7,400	3.0	9,000		
147	Pasteurized	8,230	3.1	9,300		
148	Pasteurized	10,800	4.5	13,500		
149	Pasteurized	14,400	5.1	15,300		
150	Pasteurized	7,640	3.3	9,900		
151	Pasteurized	14,800	5.3	15,900		
152	Pasteurized	9,166	4.5	13,500		
153	Pasteurized	28,060	12.6	37,800		
154	Pasteurized	1,376	0.5	1,500		
155	Pasteurized	2,216	0.9	2,700		
156	Pasteurized	9,950	4.3	12,900		
157	Pasteurized	130,000	57.0	161,000		
158	Pasteurized	12,160	4.4	13,200		
159	Pasteurized	7,100	4.0	12,000		
160	Pasteurized	9,433	3.2	9,600		
161	Pasteurized	9,430	5.2	15,600		

TABLE 1—Continued

COMPARISON OF THE HYDROXID METHOD OF COUNTING BACTERIA IN MILK WITH THE PLATE METHOD AND THE BREED METHOD

Sample of Milk, Number	Kind of Milk	Plate Method Count	Hydroxid Method Count		Breed Method Count	
		Bacteria per C.c.	Bacteria per Average Field	Bacteria per C.c.	Bacteria per Average Field	Bacteria per C.c.
162	Pasteurized	5,000	2.0	6,000		
163	Pasteurized	16,660	5.6	16,800		
164	Pasteurized	68,600	33.2	996,000	0.4	120,000
165	Pasteurized	3,730	1.0	3,000		
166	Pasteurized	1,660	0.6	1,800		
167	Pasteurized	37,700	2.4	72,000		
168	Pasteurized	5,050	1.8	5,400		
169	Pasteurized	700	0.8	2,400		
170	Pasteurized	3,513	2.1	6,300		
171	Pasteurized	13,560	4.9	14,700		
172	Pasteurized	280	0.2	600		
173	Pasteurized	5,700	3.2	4,800		
174	Pasteurized	3,360	3.8	5,700		
175	Pasteurized	8,500	6.6	9,900		
176	Pasteurized	5,860	2.5	7,500	0.1	30,000
177	Raw	97,800	40.6	121,800		
178	Pasteurized	4,420	1.9	5,700		
179	Pasteurized	7,630	2.9	7,700		
180	Pasteurized	20,000	16.9	25,000		
181	Pasteurized	4,700	2.3	6,900		
182	Pasteurized	3,860	1.5	4,500		
183	Pasteurized	14,100	3.2	9,600		
184	Pasteurized	13,200	7.3	21,900		
185	Pasteurized	6,730	2.3	6,900		
186	Pasteurized	18,730	6.8	20,400		
187	Pasteurized	6,900	2.3	7,800		
188	Pasteurized	28,330	15.5	46,500		
189	Pasteurized	47,000	16.8	50,400	0.8	240,000
190	Pasteurized	1,816	0.8	2,400		
191	Pasteurized	10,566	4.5	13,500	0.1	30,000
192	Pasteurized	10,660	5.5	16,500	0.1	30,000
193	Pasteurized	18,900	6.3	18,900	4.4	120,000
194	Pasteurized	3,800	1.9	4,800	0.1	30,000
195	Raw	2,046,000	Thousands	....	12,5	3,750,000
196	Raw	2,110,000	Thousands	....	15.5	4,650,000
197	Raw	410,000	170.1	541,280	3.6	1,146,240
198	Raw	916,000	385.8	1,225,840	5.8	1,783,000
199	Raw	1,250,000	457.0	1,371,000	5.8	1,783,000
200	Raw	670,000	282.0	897,000	7.5	2,260,600
201	Raw	733,000	306.0	919,000	4.7	1,410,000

11. Count 20 representative fields on the 2 sq. cm. and obtain the average number of bacteria per field.

12. The number of bacteria per c.c. of milk is obtained by multiplying the average number of bacteria per field by the number of microscopic fields in a sq. cm. Usually there are a little over 3000 fields per sq. cm. when the ordinary 1.8 oil immersion lens is used, but using 3000 as an even number to multiply by is satisfactory in counting.

PRECAUTIONS

If so much aluminum hydroxid precipitate is thrown down in the centrifuge that it makes too thick a film when spread over 2 sq. cm. the hydroxid suspension should be diluted. The thickness of the plug of aluminum hydroxid after centrifugalization should be about 1 mm.

TABLE 2

Determination of the Efficiency of Aluminum Hydroxid in Removing the Bacteria from Milk

Milk Sample Number	Kind of Milk	Milk before Mixing with $Al_2(OH)_6$ and Centrifuging Bacter'a per C.c.	Milk and Cream from Centrifuge Tube Mixed after Centrifuging Out the $Al_2(OH)_6$; Bacteria per C.c.	Percentage of Bacteria Removed by $Al_2(OH)_6$ and Centrifugalization
1	Pasteurized	1,220	46	96.3
2	Pasteurized	2,660	17	99.4
3	Pasteurized	20,500	210	98.8
4	Pasteurized	12,900	180	98.6
5	Pasteurized	14,900	390	97.4
6	Pasteurized	62,000	2,200	96.5
7	Raw	51,000	1,620	96.9
8	Raw	34,100	2,300	93.3
9	Raw	111,000	4,500	96.0
10	Raw	16,300	170	99.0
11	Raw	4,560	390	91.5
12	Raw	3,900	460	88.3
13	Raw	6,120	110	93.3
14	Raw	4,320	280	93.7
15	Raw	2,640	270	89.8
16	Raw	6,100	440	92.8
17	Raw	6,840	610	91.1
18	Raw	5,280	420	92.1
19	Raw	68,300	5,760	91.6
20	Raw	2,110,000	66,000	96.9
21	Raw	540,000	27,000	95.0
22	Raw	724,000	22,000	97.0
23	Raw	18,200	610	96.7
24	Raw	70,300	7,600	89.2
25	Raw	88,400	1,270	98.6
26	Raw	14,200	980	93.1
27	Raw	26,600	1,570	94.1
28	Raw	44,000	6,300	98.6
29	Pasteurized	2,740	310	88.7
30	Pasteurized	5,280	425	92.0
31	Raw	72,400	6,180	91.5
32	Pasteurized	3,460	40	98.9
33	Pasteurized	6,120	110	98.3
34	Pasteurized	49,000	2,400	95.2
35	Pasteurized	6,540	670	89.8
36	Pasteurized	1,730	86	95.1
37	Raw	44,400	310	99.3
38	Raw	13,200	140	99.0
39	Raw	63,000	5,100	92.0
40	Raw	1,185,000	69,000	94.3
41	Raw	3,170,000	264,000	91.9

No fat or toluene should appear in the film under the microscope.

Some samples of pasteurized milk show fine granular material or little curd particles due to heating. This may be removed by filtering through a small amount of cotton.

The film should stain uniformly all over. The best depth is sky blue. Too deep a stain can be moderated by dipping the film in alcohol.

Rubber stoppers must be used with centrifuge tubes, as cork will not hold the liquid in the centrifuge.

The temperature of the milk during centrifugalization should be from 25-30 C.

TABLE 3

The Determination of the Accuracy of the Hydroxid Method of Counting Bacteria by a Repetition of Counts on the Same Samples of Milk

Sample of Milk, Number	Kind of Milk	Number of Count on Same Sample of Milk	Bacteria per Field	Bacteria per C.c.
I	Pasteurized	1	8.2	25,600
		2	8.8	25,400
		3	8.7	26,100
		4	8.4	25,200
		5	9.0	27,000
		6	8.2	24,600
		7	10.6	31,800
		8	8.6	25,800
		9	10.4	31,200
		10	8.3	24,900
		11	8.0	24,000
		12	10.0	30,000
		13	10.1	30,300
		14	8.3	24,900
		15	10.5	31,500
		16	10.8	32,400
		17	9.6	28,800
		18	9.4	28,200
		19	8.0	24,000
		20	8.4	25,200
II	Raw	1	35.3	105,900
		2	34.4	103,200
		3	32.7	98,100
		4	36.3	108,900
		5	33.9	101,700
		6	35.4	106,200
		7	30.8	92,400
		8	34.9	104,700
		9	35.4	106,200
		10	32.9	98,700
		11	34.8	104,400
		12	35.9	107,700
		13	35.2	105,600
		14	37.6	112,800
		15	34.7	104,100
		16	35.2	105,600
		17	34.7	104,100
		18	36.7	110,100
		19	36.7	110,100
		20	39.9	110,700

Some practice in the technic is necessary before the best results are obtained.

COMPARISON OF THE HYDROXID METHOD OF COUNTING BACTERIA IN MILK WITH THE PLATE METHOD AND THE BREED METHOD

Data Concerning Table 1.—The samples of milk used were stirred thoroughly, then immediately plated which required about 10 minutes

for the making of the dilutions and the pouring of the agar. Then smears were made according to the hydroxid method and according to the 1/100 c.c. pipet method (Breed method).

In plating standard 1% lactose agar was used. Incubation of plates was 2 days at 37 C. and 3 days at 20 C. Triplicate plates in 3 dilutions were made of each sample. The decimal system of dilution was used.

In counting the bacteria in the hydroxid method and in the Breed method 20 representative fields were counted on each smear and the average number of bacteria per field used.

DETERMINATION OF THE EFFICIENCY OF ALUMINUM HYDROXID IN REMOVING THE BACTERIA FROM MILK

Data Concerning Table 2.—Samples of milk were plated before being mixed with aluminum hydroxid or centrifuging. Then after mixing the milk with the hydroxid suspension, shaking thoroughly, and centrifuging for 15 minutes the milk and cream were poured from the centrifuge tube into 8 c.c. of sterile water, shaken thoroughly and plated using media, and incubation time and temperature as in Table 1.

CONCLUSIONS

From the foregoing data concerning the hydroxid method of counting the bacteria in milk it is concluded that about 95% of the bacteria in the average sample of milk appear in the hydroxid thrown down by centrifugalization.

THE DIFFERENTIATION OF THE PARATYPHOID-ENTERITIDIS GROUP, III

THE UNCOMMONNESS OF B. SUIPESTIFER IN THE INTESTINES OF NORMAL SWINE

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Observations on the occurrence of bacilli of the paratyphoid-enteritidis-suipestifer group in the intestines of normal animals have been made by several European investigators, but the results and conclusions are widely at variance. The facts regarding the distribution and normal habitat of the suipestifer organisms are practically important in view of their bearing on a variety of human and animal diseases, and especially is this true if the conclusions of certain German writers in regard to the regional distribution of the group be accepted. Since the relations of these organisms to hog cholera are still obscure, the relative frequency of occurrence in normal swine offers another point of interest.

Dorset and his co-workers[1] found in one case in the intestines of normal hogs "an organism which possessed the cultural characters of B. cholerae suis," but which lacked pathogenic power for guinea-pigs. Morgan[2] also reported finding organisms having the cultural and agglutinative reactions of B. paratyphosus A and B. enteritidis in pigs' feces and in the scrapings from the mucous surface of the large and small intestines of these animals. A most comprehensive investigation of the subject was made by Uhlenhuth, Xylander, Hübener and Bohtz,[3] who examined the intestinal contents of 600 presumably healthy swine at the central abattoir in Berlin. These investigators found in 51 of these animals (8.4%) bacilli that resembled in cultural, agglutinative and pathogenic properties the bacilli that they had isolated from animals suffering from hog cholera. These observations have been extensively quoted and have been regarded as explaining the frequent occurrence of these bacilli in hog cholera as a secondary invasion of the

[1] Dorset, Bolton and McBryde, "Etiology of Hog Cholera," Bull. 72, Bur. of Animal Indus., 1905.

[2] Brit. Med. Jour., 1905, 1, p. 1257.

[3] Arb. a. d. k. Gsndhtsamte, 1907-1908, 27, p. 425.

tissues in the wake of the filterable virus. Observations by other workers have seemed to confirm this view. Grabert[4] reported finding bacilli resembling B. suipestifer in the intestines of 7 out of 23 swine without characteristic intestinal lesions, but the identification tests that he employed do not seem decisive.

Eckert[5] also isolated organisms that he considered to belong to this group from 4 out of 10 normal animals. Seiffert[6] examined the intestinal contents of 60 normal swine (Frankfurt) and found 2 cultures which "fulfilled all conditions for identification with paratyphoid-B bacilli." Schmidt[7] found bacilli "culturally similar to paratyphoid-B bacilli" in about 4% of normal swine feces (700 animals). Only one strain, however, agglutinated up to the titer limit (4,000) of the test serum ("hog cholera serum"). Other strains showed only a slight degree of agglutinability (1:200-1:800) after a number of hours in the incubator, sometimes not before 24 hours. The majority of these feebly agglutinable strains produced indol. Full details of the cultural tests relied on for differentiation are not given in this paper. Velzen[8] and Gardenghi[9] are also said to have found B. suipestifer in normal swine, but I have not been able to see their original papers for details.

Opposed to these findings is the work of Savage[10] who in a careful examination of the intestinal contents of 6 pigs failed to find organisms that could be certainly identified with true paratyphoid-enteritidis bacilli. Aumann[11] likewise obtained negative results from an examination of the intestinal contents of 101 hogs (Hamburg). Aumann states: "Upon serological examination I have found no strain which has shown even the slightest reaction toward our sera." Sobernheim[12] examined 102 samples from the intestines of 51 swine (Berlin) without a positive finding, although in 3 instances organisms which he identified as paratyphoid bacilli were isolated from the liver or spleen of the animals.

My own observations have extended over several years. The swine from which the samples were obtained were all healthy, freshly slaughtered animals in the Union Stock Yards, Chicago. The hogs

[4] Ztschr. f. Infektionskr. d. Haustiere, 1907-1908, 3, p. 218.
[5] Inaug. Diss., Giessen, 1909, cited by Hübener, Fleischvergiftungen u. Paratyphusinfektionen, Jena, 1910, p. 67.
[6] Ztschr. f. Hyg., 1909, 63, p. 273.
[7] München. med. Wchnschr., 1911, 58, p. 563.
[8] Diss. Berne, abstr. in Centralbl. f. Bakteriol., I, Ref., 1907-1908, 40, p. 802.
[9] Lo Sperimentale, 1906, abstr. in Centralbl. f. Bakteriol., I, Ref. 1907-1908, 40, p. 807.
[10] Rept. of Med. Off. to Loc. Gov't. Board, 1906-1907, p. 253.
[11] Centralbl. f. Bakteriol., I, O., 1910-1911, 57, p. 310.
[12] Centralbl. f. Bakteriol., I., Ref., Supplement, 1910, 47, p. 170.

are shipped in to Chicago from many points throughout the middle west and northwest states (about 50% from Iowa, 25% from Illinois and the remainder chiefly from Minnesota, Wisconsin, South Dakota and Indiana; some come also from Missouri and Michigan and a few from more distant points).

The samples were obtained from the lower intestine by slitting the intestinal wall with a sharp sterile knife in the manner described by Uhlenhuth.[13] The usual precautions against contamination were taken.

In the first series, obtained between Oct. 28, 1913, and April 7, 1914, the intestinal contents of 58 hogs were examined. Each sample was examined (a) by direct plating on Endo medium and (b) by preliminary incubation in brilliant green medium. The brilliant green broth was made up according to Torrey's method.[14] About 1 gm. of the intestinal contents from each hog was placed in a tube (10 c.c.) of the medium, shaken thoroughly and allowed to stand for a few minutes. From this 5 other tubes with vials to show gas production were inoculated respectively with 1 c.c. (1), 0.5 c.c. (2), 2-3 drops (2), and incubated for 24-48 hours. Those showing a positive reaction (gas production and darkening of medium) were at once plated out on Endo medium. A control tube of the medium was always carried with the others. Colonies from the Endo plates, direct (a) and after brilliant green broth incubation (b), were transferred to Russell's medium and those strains giving a paratyphoid-like reaction subjected to further study.

In all 725 colonies were transferred to Russell's medium, 343 from Endo medium direct (a) and 382 after brilliant green broth incubation (b). Of these 215 [98 (a) and 117 (b)] were inoculated into dextrose and lactose broths and gelatin. The majority of these proved of the B. coli type [151; 81 method (a), 70 method (b)], but the brilliant green incubation let through also a number of bacilli apparently of the B. proteus group [41, method (b)]. Thirteen strains were of uncertain affinities, while 10 from 6 different animals [9 isolated by method (a), 1 by method (b)] gave reactions in these media corresponding with those of the B. suipestifer type.* These 10 strains were then tested further. Only 4 of these gave milk reactions exactly corresponding to those of the paratyphoid group. None of them fermented saccharose, in this respect also resembling B. suipestifer.

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[13] Arb. a. d. k. Gsndhtsamte, 1907-1908, 27, p. 425.

[14] Jour. Infect. Dis., 1913, 13, p. 263.

* Dextrose — ; lactose — ; gelatin, not liquefied.

Dulcit was not fermented by any of the strains, but this is not a diagnostic character since, as I have previously shown,[15] the majority of typical suipestifer strains attack this carbohydrate tardily or not at all. On the other hand, only two of these fermented rhamnose and only two sorbit, one fermented salicin, and all but three produced indol. Not one of the 10 strains corresponded in cultural and biochemical characters with the suipestifer or paratyphosus type as described in a previous paper.[15]   Agglutination tests on all these organisms were made with B. paratyphosus B serums (3 strains), B. enteritidis (1 strain), and B. suipestifer (2 strains).   In no case has more than a trace of agglutination at 1 : 100 dilution been shown, although serums with high titers (5000-10,000, one B. suipestifer serum of over 20,000) have been employed.   Not a single culture in the series of 725 colonies from 58 different swine can be regarded as belonging to the suipestifer or paratyphoid type.

In the second series, March 7-May 2, 1917, specimens from 100 swine were examined.   The samples of intestinal contents were obtained in the same way and from the same source (freshly slaughtered hogs in the Union Stock Yards, Chicago) as before.   Endo medium alone was used in this series.   Three plates of three different dilutions were made from each specimen.   Paratyphoid-like colonies were transferred to agar tubes and thence inoculated into dextrose and lactose broths, litmus milk and gelatin as before.   Two hundred and fifty-three colonies were examined in this way.   Twelve from eleven different animals gave reactions in dextrose and lactose broths, and in gelatin corresponding with those of the B. paratyphosus B type.   Eight of these, however, gave reactions in litmus milk (30 days) that were unmistakably not of the Para B or Para A type.   All but one produced indol; several fermented salicin, and others failed to ferment either rhamnose or sorbit or both.

One strain resembled the suipestifer type in nearly all of its cultural characters, but failed to ferment sorbit.   All the authentic members of the suipestifer group that I have tested give a positive result with this carbohydrate.[15]   This strain did not agglutinate in 1 : 100 dilutions with potent serums of the paratyphosus A (4), paratyphosus B (12), suipestifer (63), or enteritidis (52) types.

The third series, June 14-Sept. 22, 1917, included examinations of the intestinal contents from 133 swine from the same source as before.

[15] Jordan: Jour. Infect. Dis., 1917, 20, p. 457.

The methods used were those employed in the second series. Four hundred and forty-one colonies were picked and tested. Eighteen of these from 13 different animals were dextrose +, lactose —, and failed to liquefy gelatin. None of these, however, corresponded in all their cultural characters with the suipestifer type. The lack of correlation of cultural characters in these strains is illustrated in the accompanying table.

TABLE 1

Illustrating the Cultural Deviations of Strains Tested with 5 Different Agents. The Specimens Were Taken from the Third Series of Animals

Number	Typical Reaction Milk	Indol	Salicin	Sorbit	Rhamnose
Suipestifer type * (63)	+	—	—	+	+
P 109 D	—	+	—	+	+
P 111 A	—	—	—	+	—
P 111 D	—	—	—	+	—
P 111 E	—	—	—	+	—
P 119 B	—	+	—	+	+
P 124 D	—	+	—	—	—
P 129 D	—	+	—	—	—
P 141 A	—	—	+	+	+
P 160 A	+	+	+	+	+
P 160 D	+	+	+	+	+
P 187 E	—	—	+	+	+
P 192 A	—	—	+	—	+
P 198 C	—	+	—	—	—
P 216 B	—	—	—	—	—
P 219 A	—	+	—	+	+
P 219 B	—	+	—	+	+
P 223 A	+	—	+	—	—
P 223 B	+	—	+	—	—

From the results recorded in the table it would seem not unlikely that all the mathematically possible combinations would occur in a sufficiently large series. Although only salicin, sorbit and rhamnose reactions are shown in Table 1, I have evidence that the use of other carbohydrates would increase the extent of deviation from the suipestifer type of fermentation. Some of these strains are maltose negative and behave in other ways differently from the true paratyphosus-suipestifer types.

SUMMARY AND CONCLUSIONS

In all, 1419 strains from the lower intestine of 291 normal swine have been tested. Only 40 of these have proved to be dextrose +, lactose —, and unable to liquefy gelatin.

Using only the 5 tests indicated in the accompanying table, 7 out of 40 tested gave the suipestifer reaction in milk,† 14 out of 40 did not produce indol, 22 out of 32 did not ferment salicin, 14 out of 32 fermented sorbit, and 11 out of 25 fermented rhamnose. In no instance were more than three of these five characters of the suipestifer type united in one strain. Of the 26 strains closest to the true type, 9 differed in two of the three differential tests, 7 in three and 9 in four. These strains have also been tested with paratyphoid B, suipestifer and enteritidis serums sufficiently potent to agglutinate the homologous organisms in 5,000-10,000 dilution, but not one has ever shown more than a trace of agglutination in 1 : 100 dilution.

It must be concluded, therefore, that the occurrence of true suipestifer strains in any abundance in the intestines of normal swine in this country is a rarity. The alleged frequency of occurrence of these bacilli reported from some places in Europe may perhaps be due to the inclusion of a high proportion of hogs that had become permanent or temporary carriers through their association with infected animals. Perhaps in some instances also, identification has been made on the basis of too few biologic and cultural characters. Some writers describe indol-producing strains as if they were to be differentiated hardly or not at all from typical members of the group. In the course of my examination in the past 4 years of some hundreds of typical and atypical suipestifer and paratyphoid bacilli, I have never yet found an indol-producing bacillus that did not differ in some other cultural respect or that agglutinated to any degree showing a biologic relationship with the type organisms. The observations recorded in this paper suggest that the true B. suipestifer is not a common inhabitant of the intestine of normal swine, and that its occurrence in these animals is to be looked upon as an expression of the "carrier" condition.

† Including slow production of alkalinity as in the paratyphosus-A type.

FURTHER STUDIES OF AN ANTIPOLIOMYELITIC SERUM, ITS PROTECTIVE AND CURATIVE PROPERTIES IN EXPERIMENTAL POLIOMYELITIS OF MONKEYS

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Extended observations over a considerable number of years have established the fact that one attack of poliomyelitis confers immunity against subsequent infection. Levaditi and Landsteiner[1] and Flexner and Lewis[2] have shown that the serum of recovered patients and monkeys may have acquired neutralizing and protective properties against the virus of poliomyelitis. The application of this knowledge to the treatment of poliomyelitis in man was made first by Netter[3] and more recently by others, most of whom appear to be favorably impressed with the results. Unfortunately there are several disadvantages attendant on the method of treating poliomyelitis with the serum of recovered persons. Chief among these is the difficulty in securing serum in sufficient quantities to properly treat any considerable number of cases, and the probable low antibody content of human serum.

An intensive bacteriologic study of some 250 cases of acute epidemic poliomyelitis in Chicago during the summer of 1917 has served to confirm with striking regularity the presence of the same peculiar coccus which was isolated quite constantly the previous summer both from the brain and spinal cord,[4] and also from the cerebrospinal fluid[5] in human poliomyelitis.

It was first shown by Nuzum[6] that repeated injections of the poliomyelitic coccus intravenously in animals produce antibodies in high titer, chiefly agglutinins, opsonins and complement fixation bodies. Furthermore, in a small series of experiments with monkeys, young

[1] Compt. rend. Acad. d. sc., 1910, 150, p. 131.
[2] Jour. Am. Med. Assn., 1910, 54, p. 1780.
[3] Arch. de méd. d. enfants, 1916, 19, p. 1.
[4] Mathers, George: Jour. Infect. Dis., 1917, 20, p. 113. Nuzum, J. W., and Herzog, Maximilian: Jour. Am. Med. Assn., 1916, 67, p. 1205. Rosenow, E. C., Towne, E. B., and Wheeler, G. W.: Jour. Am. Med. Assn., 1916, 67, p. 1202.
[5] Nuzum, J. W.: Jour. Am. Med. Assn., 1916, 67, p. 1437.
[6] Jour. Am. Med. Assn., 1917, 68, p. 24.

rabbits and white mice, injections of immune sheep serum appeared to confer a limited degree of protection against fatal doses of recently isolated virulent poliomyelitic cocci. Still more recently Nuzum and Willy[7] have reported very favorable results in a series of 159 cases of poliomyelitis treated with antipoliomyelitic horse serum in the Cook County Hospital during the summer of 1917.

It now appears advisable to determine the question whether or not such a serum prepared by immunizing animals with the coccus of poliomyelitis has protective or curative properties for monkeys inoculated with fatal doses of highly virulent virus. Rosenow[8] has recently reported the production of an antipoliomyelitic serum in horses immunized with poliomyelitic monkey coccus, which appears to have neutralizing, protective and curative properties against the virus.

A detailed study of the protective and curative properties of a serum produced by injection of poliomyelitic serum in experimental poliomyelitis in monkeys forms the basis of this paper.

IMMUNIZATION OF HORSE

A large horse, 6 years old, weighing 1,400 lbs., was selected and immunized, at first, according to the method described by Amoss and Wollstein,[9] which method we previously employed in the immunization of sheep. The horse received intravenous injections of living organisms in increasing doses on 3 successive days, followed by periods of rest of 7-10 days. For the first injection the 24-hour growth on a dextrose agar slant was suspended in 2 c c of sterile salt solution and 0.3 c c of this suspension in 10 c c of salt solution was injected intravenously. Twenty-four hours later 0.5 c c of the foregoing suspension of bacteria in 10 c c salt solution was injected intravenously and a similar dose given on the 3rd day. This was followed by a period of rest when a second series of injections were instituted. Small desensitizing doses were given during the first injection of each series and after the lapse of an hour the full dose was given. The organisms injected have all been originally cultured in 1% dextrose broth and later on dextrose agar slants and incubated at 35 C. for 18-24 hours. All of the strains employed for immunization have been subcultured through several generations, always under aerobic conditions, and each strain injected has been plated regularly on blood agar prior to injection into the horse.

The first serial injections were begun Jan. 30, 1917, and consisted of the poliomyelitic coccus isolated from the brain and cords of two recent human cases. The following 6 serial injections contained strains isolated from the spinal fluid and the central nervous system of 6 fatal cases of poliomyelitis, all of which were confirmed by microscopic sections of the spinal cord. The next 7 serial injections consisted chiefly of the coccus isolated from the central nervous system of 5 different monkeys recently paralyzed with virus. The

[7] Jour. Am. Med. Assn., 1917, 69, p. 1247.

[8] Ibid., p. 261.

[9] Jour. Exper. Med., 1916, 23, p. 403.

injections were given usually intravenously, but also intramuscularly and subcutaneously into the neck, shoulders and buttocks. The maximum dose consisted of the 24-hour surface growth of 3 dextrose agar slants in 8 ounce bottles. Regular serial injections of both human and monkey coccus were continued up to July 1, 1917. A test bleeding, June 6, revealed a potent immune serum. At this time 5,000 units of tetanus antitoxin were injected subcutaneously. The horse has been bled as follows: June 6, 500 c c; July 11, 2,000 c c; July 28, 2,000 c c; Aug. 8, 3,500 c c; Aug. 23, 2,500 c c.

Protection of Monkeys Against Poliomyelitis with Immune Horse Serum

It is well known that by injecting suitable doses of virulent monkey-adapted virus, the percentage of takes following intracerebral injections is 100%. In the experiments recorded in the accompanying tables we have used a moderately virulent monkey-adapted virus which was passed through 12 successive generations of monkeys in the Hygienic Laboratory, Washington, D. C., and kindly sent us by Dr. N. E. Wayson, of the Public Health Service, Washington, D. C. We passed this virus through 4 additional monkeys and used it in all of the experiments as Virus 25 (15 Generation) and Virus 27 (16 Generation). It has been injected in doses of 1 c c of a 5% emulsion which represents at least 2 fatal doses for all monkeys not immune.

In the experiments in Table 1 all of the monkeys except 53 (0.5 c c virus) received an intracerebral injection of 1 c c of the same 5% emulsion of recently glycerolated Virus 25 under ether anesthesia. Simultaneous injections of 10-20 c c immune serum intravenously 2 to 3 c c intraspinally and occasionally intramuscular injections of 10 to 15 c c of immune horse serum, were given for 2 successive days, followed by a lapse of a day, and then for 2 additional days. Monkeys 49 and 50 each received a total of 82 c c immune serum and were completely protected not only against the first injection but also against subsequent injections of virus. Monkeys 44 to 47, inclusive, each received immune serum in amounts varying from 80-110 c c. Monkeys 46 and 47 resisted the first inoculation but became severely paralyzed following the second injection of virus. Monkey 48 has no value in this series of experiments since the necropsy revealed that death was due to a traumatic intracerebral hemorrhage the 2nd day following inoculation. Monkey 52 (control) received injections of a total of 100 c c of pooled normal horse and sheep serum. He became severely paralyzed and died with typical microscopic lesions of poliomyelitis. Monkey 53 (control) inoculated with 0.5 c c of virus, developed paralysis of all extremities and died on the 4th day. It thus appears that 6 monkeys treated with immune serum were completely protected.

TABLE 1

PROTECTION OF MONKEYS AGAINST POLIOMYELITIS WITH IMMUNE HORSE SERUM
(NEUTRALIZATION OF VIRUS "IN VIVO")

Mon-key	Intracerebral Inoculation	Treatment	Result
44	1 c c Virus 25	Simultaneous intraspinal, intramuscular and intravenous injections of immune horse serum	No symptoms for 31 days. Has remained well following second inoculation
45	1 c c Virus 25	Simultaneous intraspinal, intramuscular and intravenous injections of immune horse serum	No symptoms for 21 days. Resisted a second inoculation
46	1 c c Virus 25	Simultaneous intraspinal, intramuscular and intravenous injections of immune horse serum	No symptoms for 21 days; severe paralysis 12th day following second inoculation. Recovery following injections of immune horse serum
47	1 c c Virus 25	Simultaneous intraspinal, intramuscular and intravenous injections of immune horse serum	No symptoms first inoculation; severely paralyzed 4th day following second inoculation. No improvement following injections of immune horse serum. Died 7th day
48	1 c c Virus 25	Control Injections of pooled normal horse serum	Found dead in cage 2nd day following inoculation. Necropsy revealed traumatic intracerebral hemorrhage at site of inoculation. No microscopic evidence of poliomyelitis
49	1 c c Virus 25	Simultaneous intraspinal and intravenous injections of immune horse serum	No symptoms following first inoculation. Has remained well following second and third inoculations
50	1 c c Virus 25	Simultaneous intraspinal and intravenous injections of immune horse serum	No symptoms following first inoculation. Has remained well following second and third inoculations
51	1 c c Virus 25	Control Repeated intraspinal injections of normal sheep serum	Tremor and hyperirritability 3rd day after inoculation followed by severe paralysis and death. Microscopically, typical poliomyelitis
52	1 c c Virus 25	Control Repeated intravenous and intraspinal injections of pooled normal horse and sheep serum	Symptoms 9th day; severe paralysis 10th day rapidly followed by typical respiratory paralysis and death same day. Microscopically, marked typical lesions of poliomyelitis
53	0.5 c c Virus 25	Control None	Symptoms 3rd day after inoculation rapidly followed by paralysis and death 4th day. Microscopically, typical poliomyelitis

CURATIVE PROPERTIES OF IMMUNE SERUM AFTER THE
ONSET OF SYMPTOMS OF POLIOMYELITIS

The experiments summarized in Table 2 reveal that 7 of the series of 10 monkeys treated with immune serum after the onset of poliomyelitis recovered; that is, a recovery rate of 70%. It is well known that poliomyelitis in monkeys has a death rate of from 76-100%. Five

TABLE 2

Mon-key	Inocula-tion	Symptoms at Beginning of Treatment	Treatment and Total Amount of Serum Injected	Clinical Picture after Serum	Result
25	1 c c Virus 20 intra-spinally	Tremor, ptosis, flaccid paralysis of both legs 7th day after inoculation. Eighth day monkey completely paralyzed in all extremities. Can only lift up head	44 c c immune serum injected intravenously, intraspinally and intramuscularly over period of next 5 days	Twelfth day: Tremor gone; monkey appears bright, takes food and drink eagerly. Completely paralyzed and can only move head	Survived
27	1 c c Virus 25 intra-cerebrally	Ninth day: Fine muscular tremor; flaccid paralysis of hind legs; paresis of right arm with wrist drop	60 c c immune horse serum injected as above	Tenth day: Intercostal muscles paretic; respirations rapid and labored; monkey cyanotic	Died
28	1 c c Virus 25 cerebrally	Tenth day: Flaccid paralysis of both legs; tremor and weakness of arms	80 c c immune horse serum injected as above	Thirteenth day: Tremor and fever gone; eats well; permanent paralysis of all extremities	Lived
33	1 c c Virus 27	Sixth day: General muscular weakness and stupor. Seventh day: Severely paralyzed in both legs and arms	55 c c immune horse serum injected as above	Tenth day: Appears bright; stupor gone; eats ravenously; severe residual paralysis	Lived
34	1 c c Virus 27	Sixth day: Excitable; fine muscular tremor; weak in legs	75 c c immune serum as above	Marked residual paralysis; lived 13 days after symptoms began	Favorable
54	1 c c Virus 27	Third day: Preparalytic symptoms; monkey is tremulous and very excitable. Spinal fluid clear; 330 cells per c.mm., chiefly lymphocytes. Noguchi test strongly positive	10 cc serum intravenously 3 c c immune serum intraspinally	Fifth day: Appears normal; has remained free from paralysis to date	Lived
57	1 c c Virus 27	Fourth day: Preparalytic symptoms; irritable; climbs slowly and hind legs appear weak. Spinal fluid clear; 524 cells per c.mm., chiefly lymphocytes. Positive Nonne and Noguchi	10 cc serum intravenously on 4th day. Tenth day: 22 c c of immune serum	Fifth day: Improved. Tenth day: Return of symptoms; tremor and flaccid paralysis of both legs	Lived
55	1 c c Virus 27	Third day: Preparalytic symptoms; monkey highly exciting; fine muscular tremor. Spinal fluid clear; 220 cells per c.mm., 80% lymphocytes. 20% polynuclears. Positive globulin test	12 cc serum intravenously; 2 c c serum intraspinally	Recovered; has remained free from paralysis	Recovery
56	1 c c Virus 27	Fourth day: Preparalytic symptoms; irritable and excitable. Spinal fluid opaque; 618 cells per c.mm., 80% lymphocytes. Strong Noguchi test	20 cc serum intravenously; 3 c c spinally	Has remained well to date	Recovery
58	1 c c Virus 27	Fourth day: Monkey prostrate in cage; very tremulous; flaccid paralysis of both legs	40 c c immune serum by intravenous injections	Transient improvement. Stupor and death 3rd day	Died

monkeys inoculated with virus in the usual manner received no treatment and all died within periods varying from 4 hours to 5 days after the onset of paralysis.

It will be noted that Monkeys 54 to 57, inclusive, were treated in the preparalytic stage before the onset of a flaccid paralysis but only after unmistakable symptoms and definite changes in the cerebrospinal fluid. One of this group, Monkey 57, was treated in the preparalytic stage with a single intravenous injection of 10 c c immune serum. The animal appeared to recover and remained apparently entirely normal for a period of 5 days. However, on the 10th day after the inoculation of virus the monkey developed a severe paralysis, indicating that a single injection of serum was inadequate. Recovery followed an additional injection of serum.

From Table 2 it is seen that a single dose of immune horse serum sufficed in three instances to prevent paralysis when administered in the preparalytic stage, whereas repeated doses frequently were required to save the animal's life after the onset of paralysis. Furthermore, in all of this series of monkeys except Monkey 27, injections of immune serum regularly effected a striking improvement in the clinical picture of the disease within 24-48 hours after administration. The stupor disappeared, and the fever and the muscular tremor subsided. Usually at the end of 48 hours the monkey took food and drink eagerly, although he was frequently so severely paralyzed as to be unable to lift his head from the floor of the cage. The degree of actual paralysis present at the time of treatment has always been permanent, in our experience.

IMMUNIZATION OF MONKEYS BY REPEATED INJECTIONS OF IMMUNE
SERUM PRIOR TO INOCULATION WITH VIRUS

Table 3 includes a series of 5 monkeys immunized over periods varying from 10-28 days as indicated. Monkeys 33, 34 and 37 received repeated injections of immune horse serum. Monkey 39 (control) was treated with a total of 125 c c of pooled normal horse serum. Monkey 28 (control) received a total of 90 c c of mixed normal horse and normal sheep serum over a period of 16 days prior to inoculation with virus. July 19, all of these monkeys were inoculated intracerebrally, under ether anesthesia, with 1 c c of the same 5% emulsion of glycerolated brain and cord of Virus 25. Monkeys 33, 34 and 37 proved to be immune to the first inoculation, whereas the control treated with normal serum became severely paralyzed. Further proof that infection did not occur following the first inoculation of Monkeys 33

and 34 is afforded by the fact that each of these animals became severely paralyzed following a second inoculation with virus. Monkey 37 resisted 2 different inoculations of virus but developed a severe paralysis following the third inoculation. This monkey was protected against a fatal issue by injections of a mixture of the serum of 2 paralyzed monkeys whose blood was known to possess a neutralizing effect on virus.

From Table 3 it thus appears that 3 monkeys receiving repeated injections of immune serum prior to inoculation with virus were completely protected against one inoculation of virus while the controls similarly treated but with normal serum became severely paralyzed.

TABLE 3

Immunization of Monkeys Against Virulent Virus by Repeated Injections of Antipoliomyelitic Serum

Monkey	Amount of Serum	Inoculation	Result
33 M. rhesus	June 21 to July 19: 225 c c immune horse serum injected intravenously, intramuscularly and intraspinally	July 19: 1 c c 5% emulsion of Virus 25 intracerebrally Aug. 11: Second inoculation 1 c c 5% emulsion of Virus 27 intracerebrally	No symptoms. Well 33 days after first inoculation Aug. 17, 8 a. m.: Monkey prostrate in cage; flaccid paralysis of all extremities; marked tremor. Protection after 60 c c immune serum
34 M. rhesus	July 9 to July 19: 140 c c immune horse serum by intravenous, intramuscular and intraspinal injections	July 19, noon: 1 c c 5% emulsion of Virus 25 Aug 11, noon: 1 c c 5% emulsion of Virus 27	No symptoms 23 days after first inoculation Aug. 17, 1 p. m.: Paralyzed in hind legs; weakness of right arm; improvement following 75 c c immune horse serum
37 Ring Tail	July 13 to Aug. 2: 123 c c immune serum injected as above	Aug. 2: 1 c c 5% emulsion of Virus 25 Aug. 11, a. m.: 1 c c 5% emulsion of Virus 27 Aug. 22, noon: 1 c c 5% emulsion of Virus 27	No symptoms Remained well Aug. 25: Tremor and excitability followed by flaccid paralysis of both legs. Aug. 27: Protection following intravenous and intraspinal injections of immune Monkey Serums 16 and 18
Control 39 Ring Tail	July 10 to July 19: 125 c c pooled normal horse serum injected intravenously, intraspinally and intramuscularly	July 19, 1:30 p. m.: 1 c c 5% emulsion of Virus 25 intracerebrally	July 22: Onset of symptoms rapidly followed by paralysis and death July 24. Microscopically, typical lesions of acute poliomyelitis
Control M. rhesus 28	90 c c of mixed normal horse and normal sheep serum injected intravenously, intramuscularly over period of 16 days	1 c c Virus 25 5% emulsion intracerebrally	Symptoms 9th day after inoculation; 10th day severely paralyzed in all extremities; stuporous. Recovery followed injection of 80 c c immune horse serum. Living 51 days later with marked atrophy of all extremities

The Opsonic Content of Immune Horse Serum and of Serum of Paralyued Monkeys

Over 80 estimations have been made of the opsonic content of immune horse serum and of the serum of paralyzed monkeys for different strains of the poliomyelitic coccus isolated both from human and monkey sources. In this work we have used the dilution method to determine at what point induced phagocytosis definitely exceeded spontaneous phagocytosis. The washed leukocytes of the same normal adult person have been used in all of these determinations.
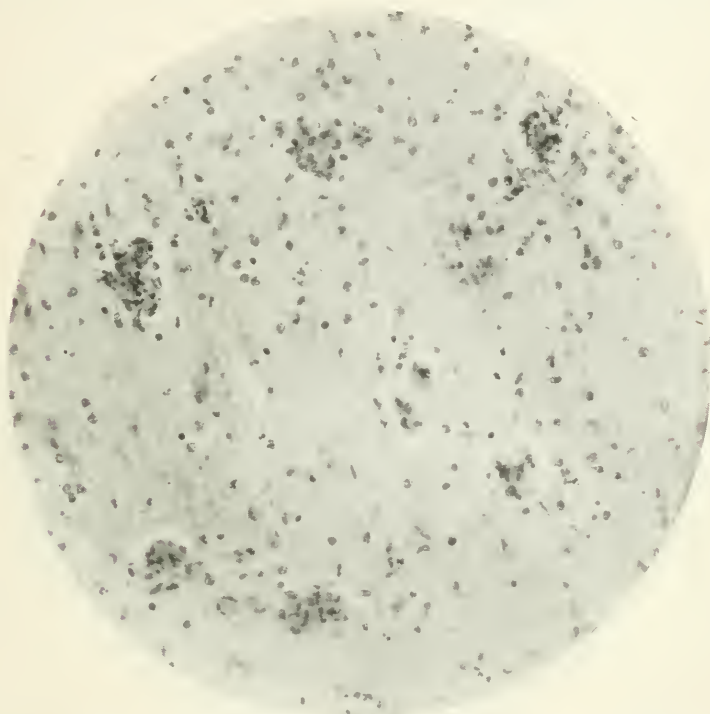
Fig. 1.—Illustrating typical neurophagocytosis in the ganglion cells of the lumbar level of the spinal cord of Monkey 27. This monkey was a control inoculated with virus in the usual manner and treated with a total of 90 c c of pooled normal horse and sheep serum. × 230.

The degree of spontaneous phagocytosis was estimated by the amount of phagocytosis in specimens of salt solution instead of serum. In these experiments we used typical strains of poliomyelitic cocci isolated from the spinal fluid of patients both before and after death, from the central nervous system of fatal human cases and also from the brain and cord of paralyzed monkeys.

As controls and to determine the specificity of the reaction, we used 2 strains of Streptococcus viridans from cases of acute endocarditis, a hemolytic streptococcus, 10 strains of streptococci from scarlet fever, 1 strain of pneumococci and 2 strains of Staphylococcus aureus and Staphylococcus albus. Suspensions of

the organisms were prepared by washing off the 24-hour surface growth of an agar slant and diluting with saline solution to approximately the same turbidity. After incubation of the tubes at 35 C. for 30 minutes, 50 polynuclear leukocytes were counted and the number of cells engaged in phagocytosis determined.

The point of opsonic extinction of the immune horse serum as estimated with homologous strains of poliomyelitic cocci has reached 1 : 6,000 both for human and monkey strains of cocci. Moreover, by
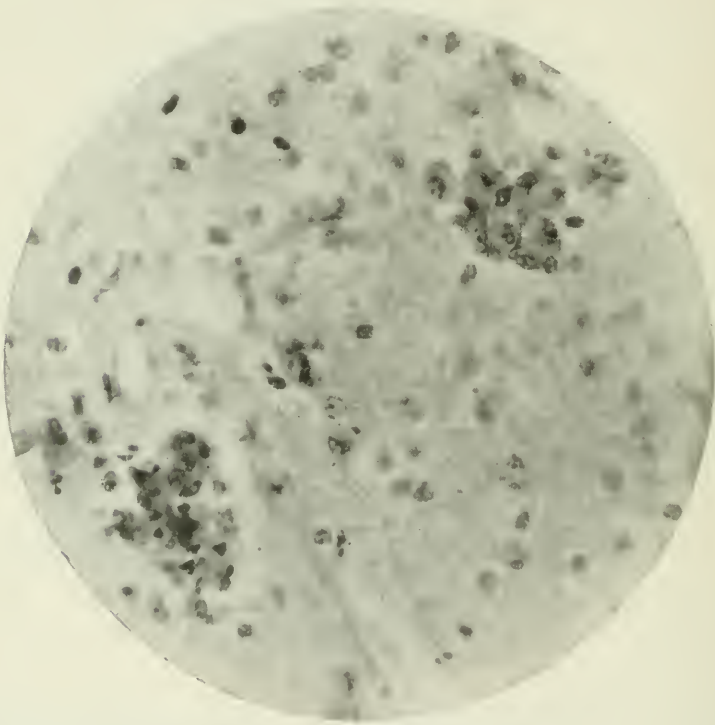
Fig. 2.—Same as Fig. 1.   × 500.

means of immune horse serum we have been able in 20 instances to identify and separate strains of poliomyelitic cocci recently isolated from the spinal fluid of man from other strains of cocci presenting more or less confusing cultural and morphologic similarities. Further-more, the serum of 6 paralyzed monkeys recovering after treatment with immune horse serum have all shown a high specific opsonic con-tent both for human and monkey strains of the poliomyelitic coccus.
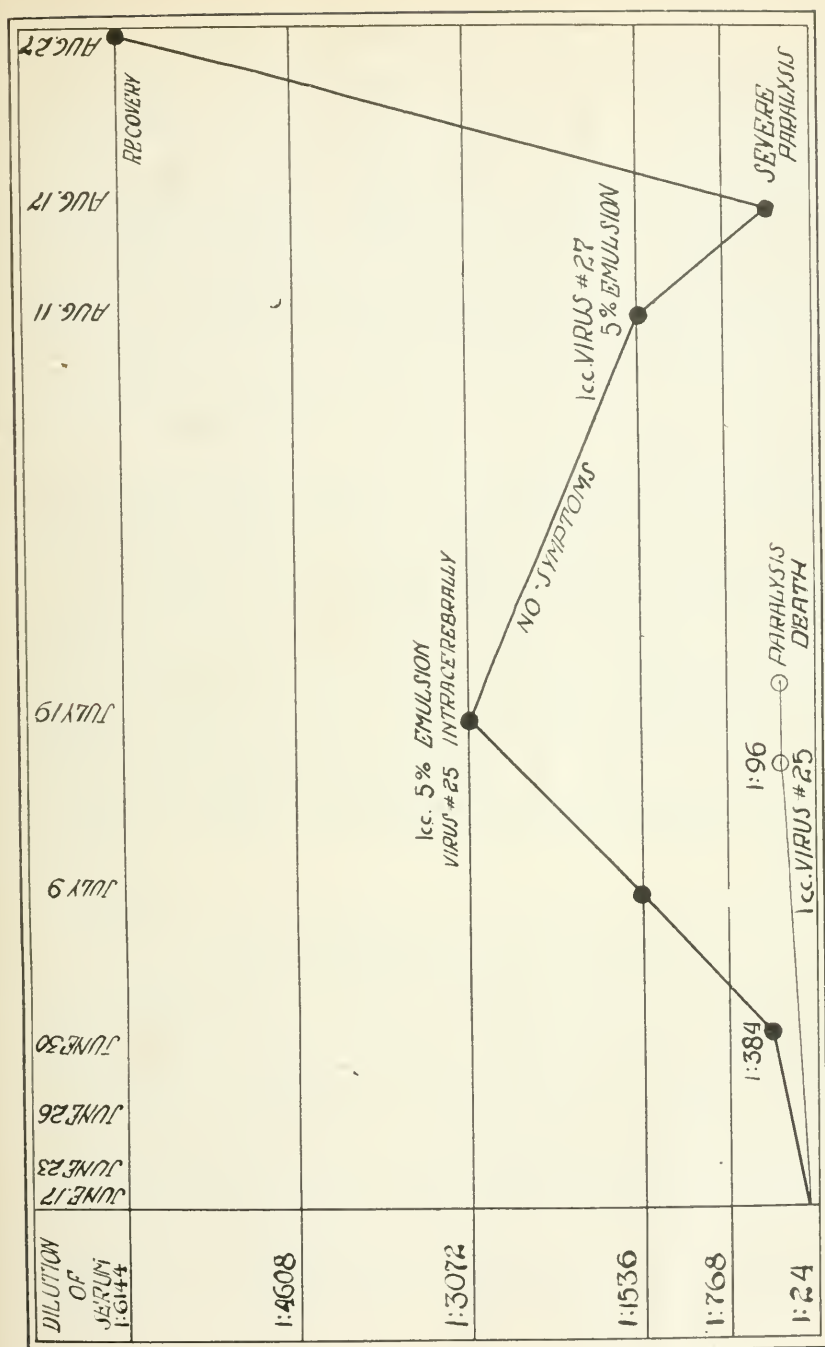
Chart 1.—The rise in the opsonin content of the serum of a monkey receiving injections of immune horse serum. Note the corresponding fall in the opsonic titer following injections of virulent virus.

Mathers and Tunnicliff[10] have shown a specific increase in the opsonin content of the serum of convalescent children for the coccus of poliomyelitis.

The heavy line on Chart 1 illustrates the rise in the opsonin content of the serum of Monkey 33, (M. rhesus) following repeated injections of immune horse serum. The light line represents the point of opsonic extinction for the poliomyelitis coccus in the serum of a control monkey treated with injections of pooled normal horse serum. Injections of immune serum were given intravenously, intramuscularly and intraspinally as follows: June 17, 50 c c serum; June 23, 25 c c; June 26, 44 c c ; June 30, 35 c c; July 10, 75 c c immune horse serum. July 19, the opsonin titer of the serum of Monkey 33 was 1:3072, while the point of opsonic extinction for the poliomyelitic coccus in the serum of the control monkey was but slightly above normal, that is, 1:96. At this time each monkey received an injection of Virus 25 intracerebrally. The control monkey became severely paralyzed and died the 4th day after inoculation. Monkey 33 was immune and after a lapse of 3 weeks the opsonin content of his serum had fallen to 1:1,536. At this time, Aug. 11, he received a second inoculation of Virus 27 and became severely paralyzed in all extremities on the 6th day. This monkey recovered following injections of 60 c c immune horse serum. Ten days after the onset of the paralysis and at a time when the animal had been actively immunized against subsequent injections of virus, the opsonic content of the serum had arisen to 1:6,144.

IMMUNIZATION OF MONKEYS BY INTRACEREBRAL OR INTRAVENOUS INJECTIONS OF THE POLIOMYELITIS COCCUS

We wish now to record briefly the details of an experiment which indicates that a single intracerebral injection of a markedly attenuated culture of the poliomyelitic coccus, originally isolated from the spinal fluid of a patient during life, produced immunity in a monkey to repeated injections of virulent virus. Two additional monkeys immunized with a single intracerebral injection and repeated intravenous injections of attenuated cultures of poliomyelitis coccus isolated from both human and monkey sources, have been rendered immune to repeated inoculations of several fatal doses of highly virulent virus. The details of these experiments are summarized in the protocols

[10] Jour. Am. Med. Assn., 1916, 67, p. 1935.

which follow. In this connection it seems important to refer briefly to the recent work of Amoss[11] on the immunization of monkeys with cultures of the globoid bodies. Eleven monkeys received regular weekly injections of mass cultures of 4 strains of the globoid bodies over a period of 2 years. Some were injected intracerebrally, others intravenously, intraspinally and intraperitoneally. None of these monkeys developed poliomyelitis and their serum apparently did not acquire neutralizing properties against the virus of poliomyelitis. Amoss concludes that the response to antibody formation is small and at most leads to but slight reactions of agglutination and complement deviation with the cultures.

Exper. 1.—Monkey 16 (M. rhesus). April 13, 1917: Monkey etherized and given an intracerebral injection of 1 c c of a heavy suspension of mixed subcultures in the fourth generation originally isolated from the spinal fluid of patents with poliomyelitis. These strains had been cultured in the laboratory for a month and grew as fine green colonies on blood-agar plates.

May 7: Well. An intravenous injection of the surface growth of 3 agar slants of a third generation culture of the coccus from monkey Virus 312 was given.

May 21: An intravenous injection of the 24-hour surface growth of 5 agar slants of a second generation culture of the coccus isolated from monkey Virus 22 was given.

June 15: The monkey was etherized and given an intracerebral injection of 1 c c of a 5% emulsion of Virus 25.

July 19: The monkey has remained well.

July 19: Etherized and given a second intracerebral injection of 2 c c of a 5% emulsion of recently glycerolated brain and cord of monkey Virus 25 (four fatal doses).

Aug. 30: The monkey has remained well; has never shown any signs of poliomyelitis.

Exper. 2.—Monkey 35 (Ring Tail). June 27: Etherized and given an intracerebral injection of the poliomyelitis coccus isolated during life from the cerebrospinal fluid of E. H. The coccus was originally isolated in a tube of 1% dextrose broth inoculated with 1 c c of clear spinal fluid. Subcultures on standard human blood-agar plates grew as fine, slightly green colonies. The original coccus was carried through three blood-agar plates with each transplant being made from a single colony fished from the agar medium. The third generation on blood agar was inoculated into dextrose broth and subcultures were then made on agar slants. The surface growth of three agar slants was suspended in 1.5 c c of sterile salt solution and this sixth generation culture of the coccus was injected into the left cerebral hemisphere of Monkey 35.

July 19: The monkey has remained well. Was given an intracerebral injection of 1 c c of 5% emulsion of Virus 25.

Aug. 11: No symptoms to date. Etherized and given a second intracerebral injection of 1 c c of 5% emulsion of Virus 27.

Aug. 30: The monkey has never exhibited any symptoms of poliomyelitis.

[11] Jour. Exper. Med., 1917, 25, p. 545.

Exper. 3. Monkey 18 (M. rhesus). April 20: Etherized and given an intracerebral injection of 1 c c of the sedimented broth culture of Virus 25 now in the fourth generation.

May 7: Has remained well. An intravenous injection of the surface growth of 3 agar slants of a third generation culture of the coccus from monkey Virus 312 was given.

May 21: Given an intravenous injection of the surface growth of 4 agar slants of a fifth generation culture of the coccus from Virus 22.

June 15: Etherized and given an intracerebral injection of 0.5 c c of a 5% emulsion of Virus 25.

Aug. 2: No symptoms. Etherized and given a second intracerebral injection of 1 c c of a 5% emulsion of Virus 25.

Aug. 30: Monkey has remained well.

Exper. 4. Monkey 30 (M. rhesus). June 15: Etherized and 1 c c of 5% emulsion of Virus 25 injected intracerebrally.

June 19: Tremor, irritability and convulsions.

June 20: Flaccid paralysis of both legs and weakness of the arms. Died during the night. Microscopically, typical lesions of poliomyelitis.

SUMMARY

A highly potent immune serum can be produced in the horse by repeated intravenous injections of aerobic cultures of the poliomyelitic coccus.

Such a serum contains agglutinins, complement fixing bodies and especially opsonin in high titer.

Monkeys can be immunized against several fatal doses of virulent monkey-adapted virus by repeated intravenous and intracerebral injections of the poliomyelitic coccus.

The serum of the horse immunized with strains of the poliomyelitic coccus obtained from human and monkey sources possesses protective and curative properties against the virus in experimental poliomyelitis of monkeys.

THE EFFECT OF HIGH PRESSURES ON BACTERIA

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.The work recorded in this paper is the result of an attempt to study the mechanism of immunity and incidentally to test a theory regarding the failure of vaccine therapy as practiced at the present time.

During the past 8 or 10 years bacterial vaccines have been used extensively, but, on the whole, with very unsatisfactory results. Were it not for the excellent theoretical foundation on which vaccine therapy rests, its use would probably have been relegated to the past. Bacterial vaccines, as used today, judged by the practical results obtained, are of little or no value aside from typhoid vaccine as used prophylactically and the undisputed value of staphylococcic and B. acne vaccines, in certain types of cases.

Protective immunization experiments with killed cultures on laboratory animals have likewise not been crowned with marked success. Facts such as these may readily be gleaned from a survey of the literature. The conclusion would therefore seem justified that the dead organisms do not offer a suitable antigen for immunization processes.

During the past year while conducting some perfusion experiments on rabbits, we were much impressed by the rapidity with which bacteria were taken up by phagocytes. The particular experiment in question concerned the perfusion of the liver of rabbits with emulsions of staphylococci. By inserting cannulae in the portal vein and superior vena cava the liver could readily be perfused with Locke's fluid, containing large quantities of staphylococci in suspension. It was found that a fluid containing 9,000,000 organisms per cubic centimeter could be sterilized in a few minutes by being passed through this organ. The endothelial cells of the liver, on section, were found to be literally packed with bacteria following this operation. This observation suggested that vaccines injected into an individual for the purpose of

immunization probably meet a similar fate. The phagocytes, whose function it is to seize any foreign material entering the body, failing to recognize the benevolent intention of the immunizer, probably take up the killed cultures with even more greed than the living bacteria. Metchnikoff[1] and his pupils have shown that the digestion of such incorporated particles by phagocytes may be very rapid, especially when it concerns dead bacteria.

It is a well recognized fact that an antigen which has undergone even partial digestion is no longer capable of stimulating antibody formation, and hence ceases to be an antigen. The natural deduction is therefore to assume that phagocytosis defeats, in a measure at least, attempts to immunize with killed bacterial cultures. Metchnikoff and his school would have us believe that the phagocytes are the source of antibody formation, but the evidence presented in support of this contention is far from conclusive.

If we now examine antigens, such as diphtheria and tetanus toxins, which are not in the form of particles, but in a diffused state and hence not so accessible to the phagocytes, there is little difficulty in attaining a high degree of immunity. An animal may likewise be given a few injections of foreign protein, such as egg white, with the result that a high titer of immune serum is formed. The exception to this rule would seem to be the immunization with tissue cells and red corpuscles in particular. A few injections of foreign red cells will usually give a serum with a very high lytic titer. This may, however, be explained by the fact that tissue cells are taken up by the so-called macrophages, which are large cells with a rigid nucleus, and which are slow to arrive on the field of action. It is not improbable that the antigenic principle is dissolved from the injected cells by the tissue fluids before phagocytosis takes place. Prompted by these considerations an attempt was made to extract the antigenic principle from bacteria in a form not so readily accessible to phagocytes in the hope that it might give vaccine therapy the much needed "raison d'être."

The conversion of bacteria from the solid or semisolid state to a state of diffusion is not an easy matter, owing to the firm outer membrane. Numerous investigators have subjected bacteria to various physical influences looking to this end, such as high pressure, shaking, freezing and grinding processes, but hitherto with little or no success.

E. Büchner[2] was the first to obtain definitely positive results by subjecting living cells to high pressure. By the use of a hydraulic press he was able to

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[1] Metchnikoff: L'Immunite, 1901
[2] Buchner, E.: Ber. d. deutsch. Chem. Ges., 1897, 30, 117.

separate zymase from the yeast cell.   Later, Hans Büchner,[3] by using the same apparatus, subjected bacteria to a pressure of 500 atmospheres in an attempt to isolate ferments and endotoxins, but with rather disappointing results. Certes[4] found that a pressure of 350 to 500 atmospheres had no effect on putrefactive processes.   Yeast cells under a pressure of 400 atmospheres were still able to ferment sugar.   The same investigator found that the pathogenicity of anthrax bacilli was not impaired after having been subjected to a pressure of 600 atmospheres.   D'Arsonval[5] and Charrin on the other hand found that CO_2 under a pressure of 50 atmospheres destroyed B. pyocyaneus in 2 hours.   Their work was repeated by Sabruzes[6] and Bazin, Schaeffer[7] and Freudenreich, and Krause,[8] all of whom failed to confirm the findings of the French investigators. In summing up the results of high pressures on bacteria Gottschlich[9] concludes that they have little or no effect.

In our work many pieces of apparatus have been constructed, but only those with which definite results were obtained will be described. We first studied the effect of a direct load on bacteria.

For this purpose a piece of nickel-chrome-steel was obtained from one of the large steel companies, which also kindly furnished us with an analysis of the material.   A hole $7/8$ inch in diameter was bored through a piece of steel 5 inches by $3\frac{1}{2}$ inches, and a piston, likewise of nickel-chrome-steel, was ground to fit the cylinder.   One end of the cylinder was closed by a steel wedge. The cylinder, piston, and wedge were then tempered to withstand a pressure of 225,000 lbs. per square inch.   The bacteria that were to be subjected to pressure were mixed with infusorial earth, after which they were placed in a leaden cup about 2 inches long and of a bore to fit the steel cylinder above described.   The leaden cup, about two-thirds filled, was placed in the cylinder in an inverted position and forced to the bottom of the cylinder where it rested on a leaden disk which, in turn, rested on a steel wedge closing one end of the cylinder.   The piston was then inserted and the apparatus placed in a testing machine such as is used by engineers in testing the strength of materials.   The particular machine in question was capable of lifting a load of 200,000 lbs.   The tests were usually inaugurated at 6 p. m. and terminated 14 hours later.

The following organisms have been studied in this manner: B. typhosus, B. coli, B. tuberculosis, B. proteus, B. subtilis, staphylococci, streptococci and pneumococci.

The pressures used ranged from 3,000-12,000 atmospheres.   On termination of each experiment cultures were made on dextrose broth and, whenever possible, plates were made before and after the experiment.   The general statement may be made that a pressure of 3,000 atmospheres was not sufficient to destroy any of the bacteria studied,

[3] Buchner, E.: München. med. Wchnschr., 1897, 44, 299.
[4] Certes: Compt. rend. Acad. de Sc., 1889, 99, 385.
[5] d'Arsonval & Charrin: Compt. rend. Soc. Biol., 1893, 467, 764.
[6] Sabrazés & Bazin: Koch's Jahresb. f. Gärungsorganism, 1893, 34.
[7] Schaffer & Freudenreich: Ann. d. Micrographie, 1890, 4, 502.
[8] Krause: Centralblatt f. Bakt., etc., 1902, O., 31, 673.
[9] Gotschlich: Kolle-Wassermann Handb. d. path. Mik., 2 Aufl., 3, 460.

while a pressure of 6,000 atmospheres for 14 hours was found to destroy all non-spore-forming organisms. The spores of B. subtilis were not regularly killed at pressures of 12,000 atmospheres. The bacteria would undoubtedly have been destroyed by these pressures in less time than that used; further studies are being undertaken to determine this point, however.

Having thus determined that it was possible to destroy bacteria by physical pressure, the question naturally arose as to the mechanism operative in this procedure. It was probably not a question of expressing the cytoplasm from the organisms, as was attempted by Büchner, inasmuch as the pressure exerted on the bacteria was uniform from all sides. The small amount of entrapped oxygen in the container could scarcely be responsible for the phenomenon. It was also felt that the element of heat development could be ruled out without further consideration. Two other factors were still to be considered: the direct effect of the pressure, and its sudden release. Which of these two was the important factor was, by the very nature of the experiment, difficult to determine, but it was not improbable that both factors played an important rôle in killing the bacteria. The interesting observation was made that bacteria subjected to high pressure were difficult to stain; as a rule only shadows could be discerned where it concerned gram-negative organisms, while the gram-positive ones usually lost their specific staining reaction.

It would seem probable from these experiments that the death of the bacteria was due either directly to the pressure or its sudden release or to a possible combination of both.

In an effort to analyze more fully some of the problems which had presented themselves during the process of these investigations, studies were inaugurated to determine the effect on various bacteria of gases under high pressure, such as CO_2, H, and N. An apparatus was constructed for this purpose which would enable us to subject bacteria to various gas pressures and suddenly release it. The apparatus used for these experiments may briefly be described as follows:

A steel tube, 8 inches in length and 1½ inches in diameter, lined with block tin, was attached to the desired gas tank by a suitable coupling. The opposite end of the apparatus was fitted to a brass head containing a small receiving chamber. The bacteria to be studied were placed in a sterile test tube resting on a coiled spring in the bottom of the tin-lined steel tube. The receiving chamber was provided with a valve, connected with a glass tubing which extended to the bottom of the test tube containing the bacteria. The apparatus was then attached to the gas tank and left for the desired time. When the

valve leading to the receiving chamber was opened, the pressure of the gas forced the bacterial suspension up the glass tube, through the valve into the receiving chamber. The fluid so driven into the receiving chamber could be collected from a small spout into a sterile test tube. It will thus be seen that the pressure was released suddenly as the fluid passed the valve. The valve was under such control as to permit the removal of the fluid drop by drop if desired. We are indebted to Mr. David Crowther, Mechanician and Instructor in Dental Mechanics, University of Minnesota, for valuable mechanical assistance. We are indebted also to Dr. J. F. McClendon for helpful suggestions.

It was found that CO_2 under a pressure of 50 atmospheres would destroy B. typhosus, B. coli, B. tuberculosis, B. pyocyaneus, staphylococci, streptococci, and pneumococci in a period of time ranging from $1\frac{1}{2}$-$2\frac{1}{2}$ hours. It was interesting to observe that the gram-negative bacilli could be brought to a marked degree of disintegration, although disintegration of all the bacteria in suspension was never attained. The gram-positive cocci, on the other hand, suffered little morphologic change aside from slight irregularity in size, and often a tendency to lose their gram-positive character. The importance of suspending bacteria in distilled water instead of broth or physiologic salt solution was emphasized by the fact that when the latter were used very little disintegration of the organisms was noted. It was found that CO_2 of less than 40 atmospheres produced no effect whatever on the bacteria studied. Yeast cells were found to be unaffected by CO_2 after exposure to this gas for 48 hours. This may be due to the fact that yeast cells are normally active CO_2 producers, and their external membrane therefore probably offers little or no resistance to the CO_2 molecule. The osmotic equilibrium of these cells was probably not disturbed by the sudden change in the molecular concentration of the fluid. CO_2 likewise has no effect on the proteolytic ferments of B. pyocyaneus and B. proteus; these ferments were found to be as active after exposure to this gas at 67 atmospheres for 20 hours as were the controls kept at room temperature.

Having then found that bacteria could be destroyed by relatively low gas pressure, we were once more confronted with the problem as to the mechanism of the various physical and chemical forces involved in the experiment.

It seemed probable that the acidity of the CO_2 solution under the pressure used might be responsible for the killing of the organisms, although their disintegration was evidently due to the sudden expansion of the dissolved gas. Being unable to find any work dealing with the degree of acidity of CO_2 solutions under pressure, experiments to

determine the H ion concentration of this gas under various pressures were undertaken. The methods suitable for this particular experiment were, owing to its peculiar nature, limited. The colorimetric method seemed to be the most feasible. For this experiment a steel tube 4 inches long and 1 inch in diameter was lined with pure block tin. The ends of the tube were closed with glass lenses, supported further by heavy steel burrs. The indicator to be used was then placed in this apparatus which by means of a suitable coupling was attached to the CO_2 tank. Any change in the indicator could thus readily be determined. In order to be able to detect even a slight change in the indicator it was found necessary to construct a duplicate apparatus always containing the same indicator which could be used as a control. The P_H was found to be between 10-3 and 10-4, though probably nearer 4 than 3. It was found that the amount of pressure used had very little effect on the degree of acidity; in fact, experimentally, it was difficult to show any difference in the degree of acidity between 1 and 50 atmospheres' pressure, although theoretically the P_H of CO_2 at 50 atmospheres is 10-3.15 and at 1 atmosphere 10-3.6. Our failure to establish any difference in the degree of acidity of this gas under various pressures was probably an insufficient number of indicators of the proper range were used.

The next logical step in this series of experiments seemed to be to determine the effect of this degree of H ion concentration on the bacteria studied. Hydrochloric acid, acetic and phosphoric acids were prepared to a H ion concentration of 10-3.15, the maximum theoretical acidity of the CO_2 used. It was found that B. coli and the staphylococcus would live from 7-10 days in the hydrochloric and acetic acid of this acidity. The phosphoric acid (which is not buffered appreciably by proteins) was found to be more injurious to the organisms. However, both the staphylococcus and B. coli survived the action of the phosphoric acid for 48 hours. Our findings in this respect do not coincide fully with those of Michaelis,[10] who found that B. coli was destroyed in 48 hours in a solution of lactic acid at a P_H of 10-4. It is not improbable that various strains of the same organism may vary in regard to their resistance to acids.

These experiments seem to justify the conclusion that the acidity of the CO_2 is not an important factor in the destruction of the organism. It therefore seemed probable that the high molecular concen-

[10] Michaelis: Ztschr. f. Immunitätsforsch., 1912, O., 14, 170.

tration or the sudden change in the osmotic tension of the fluid, or possibly both, were the factors which destroyed the bacteria. CO_2 is very soluble in water, 0.8 of a volume being soluble for each atmospheric pressure. The maximal CO_2 pressure used in our experiments was 67 atmospheres, which, according to the law of the solubility of gases, would cause 52 volumes of CO_2 to go into solution. In order to determine the effect of this molecular tension on bacteria a solution of NaCl of corresponding concentration (7.2%) was prepared. Staphylococci and B. coli were suspended in this solution, which was acidified to a point of P_H 10-3.15, and cultures were taken hourly. It was found that these organisms were killed in about 15 hours. It does not follow, naturally, that, because the solution of CO_2 and sodium chlorid were of the same molecular concentration, their effect on bacteria would be identical. The effect of each on the membrane of the bacteria might be widely different; possibly the size of the molecule might be a vital factor, not to mention the possibility of the influence of incidental chemical reactions. Ignoring, for argument's sake, these unknown factors, and assuming the effect of the two solutions on bacteria to be much the same, we are led to the conclusion that the sudden release of the pressure, thereby lowering the osmotic tension of the fluid, is the vital factor in killing the bacteria, as it was found that bacteria were killed in from $1\frac{1}{2}$-$2\frac{1}{2}$ hours in CO_2 solutions, whereas 15 hours were necessary to kill them in acidified salt solution of the same molecular tension. The $1\frac{1}{2}$-$2\frac{1}{2}$ hours probably represents the time required for the gas to become dissolved in the water and to diffuse through the bacterial membranes.

The effect of hydrogen on bacteria was likewise studied. Hydrogen being only slightly soluble, it was not expected that its effect would be so marked as that of CO_2, its solubility being 0.031 volumes per atmosphere. Bacteria were placed in the apparatus described in the previous experiment and attached to a hydrogen tank under pressure of 120 atmospheres. It will thus be seen that only 2.2 volumes of hydrogen could be dissolved as compared with 52 volumes of CO_2. Sterile cultures were never obtained under the influence of hydrogen, although by plating the bacteria before and after treatment it was possible to demonstrate that from 10-40% of B. coli had been killed in 24 hours. There was evidence that many of the bacteria were broken up under the influence of hydrogen and this seemed in particular to be true of the tubercle bacilli. The most marked effect of

hydrogen on bacteria, however, was its tendency to affect their staining reaction. Gram-positive organisms would often become gram-negative, and even the acid-fast character of the tubercle bacilli was impaired.

A study was made of the effect of hydrogen on the pathogenicity of pneumococci; it was found, however, that the pathogencity of these organisms for white mice remained unaltered.

Nitrogen under a pressure of 120 atmospheres seemed to be entirely inert. The bacteria were not killed; neither did they present any morphologic change.

IMMUNIZATION EXPERIMENTS

As stated at the beginning, our object was to obtain the antigenic principle of bacteria in a diffused state in order to make it less accessible to the phagocytes, thereby hoping to obtain an antigen capable of producing a higher degree of immunity than is ordinarily possible with bacterial antigens. Typhoid bacilli which had been under orthostatic pressure of from 6,000-12,000 atmospheres were placed on a shaking machine for 10 hours, after which they were filtered through a Berkefeld filter and the filtrate injected into rabbits.

The question of a standard dosage at once became a serious problem. Bacteria which had been under pressure were difficult to standardize. The following technic was therefore adopted. Six 24-hour slant agar cultures were mixed with infusorial earth and placed under pressure for 14 hours. On removal from the pressure apparatus the infusorial-earth-bacterial mixture was taken up in 50 c c of distilled water and placed on the shaking machine and later filtered. Two c c of the filtrate were then injected into the marginal ear vein of rabbits at 5-day intervals. Ten days following the third injection the agglutinating titer of the blood serum was determined. It was not uncommon to find that the serum of rabbits thus treated would agglutinate typhoid bacilli in dilutions of 1 : 10,000. We rarely found the titer to be below 1 : 5,000. Rabbits treated with living typhoid bacilli in the same manner never yielded a serum of this agglutinating titer: from 1 : 100 to 1 : 500 being the average agglutinating titer of the serum of rabbits treated in this way.

Animals were likewise immunized with bacteria and bacterial filtrate of cultures killed by CO_2. Although these experiments are not yet terminated it may be said that bacteria killed by CO_2 give excellent results in the way of stimulating antibody formation, it being found that typhoid bacilli killed in this way gave an agglutinating serum of a

higher titer than when a corresponding amount of the living culture was used. The B. coli as well as other gram-negative bacilli, it was observed, became far more toxic after having been killed by CO_2 than the living cultures. This is probably due to the fact that large amounts of bacterial protein were liberated by the killing process.

A few attempts to immunize with the hydrogen-treated bacteria have been undertaken. In studying the complement-fixation reaction in tuberculosis excellent results were obtained with antigens which had been disintegrated by hydrogen.

RÉSUMÉ

It has been found that a direct pressure of 6,000 atmospheres kills non-spore-forming bacteria in 14 hours. A pressure of about 12,000 atmospheres for the same length of time is required to kill spores.

Non-spore-bearing bacteria are killed by CO_2 of 50 atmospheres pressure in about 1½ hours. Yeast cells withstand the action of CO_2 for more than 24 hours, probably because of their ability to transmit the CO_2 molecule through the membrane promptly.

The maximum acidity of solutions of CO_2 used in our experiments was P_H 10-3.15. It was shown that this degree of acidity was not a factor in killing the bacteria. A solution of sodium chlorid of a molecular concentration corresponding to a solution of CO_2 under 67 atmospheres and acidified to P_H 10-3.15 required 15 hours to render cultures of staphylococci and B. coli sterile. It was therefore concluded that the sudden change in the osmotic tension of the fluid in which the bacteria were suspended was the factor which destroyed the organisms.

Many of the gram-negative bacteria could be broken up by the sudden release of the CO_2 while the gram-positive bacteria, although killed, suffered little or no morphologic change.

Hydrogen killed from 10-40% of B. coli. This gas had no effect on the pathogenicity of pneumococci.

The acid-fast character of the B. tuberculosis was impaired by the action of hydrogen. Hydrogen-treated tubercle bacilli yielded a suitable antigen for the complement fixation reaction.

Nitrogen under a pressure of 120 atmospheres had no effect on bacteria.

Filtrate of typhoid bacteria which had been subjected to a direct load of 6,000 atmospheres for 14 hours was found to be far superior to the living culture as an immunizing antigen. Bacteria killed by CO_2 were likewise found to be excellent antigens.

THE ETIOLOGY OF EPIDEMIC POLIOMYELITIS

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A somewhat peculiar diplococcus or streptococcus has been found in the spinal fluid, and in the brain and cord of cases of poliomyelitis by various observers. In some instances injections in animals gave suggestive, although inconclusive results indicating the causal relationship of this organism. Other workers were unable to obtain corroborative evidence. Efforts to demonstrate bacteria in sections proved unsuccessful. The discovery that typical poliomyelitis can be produced in the monkey with so-called virus and filtrates of virus,[4, 9, 10] and the successful inoculation of monkeys by Flexner and Noguchi with the small "globoid" organism which they cultivated from the central nervous system in poliomyelitis, was considered final in proving that bacteria of ordinary size had no etiologic relation to this disease. The status of the question of the relation of these organisms, particularly streptococci, to poliomyelitis, when we began our studies is tersely stated by Wickman: "Such bacteria must be regarded as having had an accidental and not a causal relation to the malady."

However, since the earlier studies were made, special methods have been developed and bacteria of ordinary size have been isolated from tissues which were considered sterile. The localization of these bacteria in animals frequently corresponded closely to that in the disease from which they were isolated. In view of these results, the older investigations were considered inconclusive as proving that bacteria of ordinary size had no etiologic relationship to poliomyelitis, and a restudy of the bacteriology of poliomyelitis by the use of the newer methods was undertaken.

We wish now to record the details of a study of a series of cases of poliomyelitis which occurred in Rochester, Minnesota, and New York City during the epidemic of 1916, and in Davenport, Iowa, in 1917. The results of the cases occurring in 1916 have been described in a preliminary report[19] in which the following statement was made:

TABLE 1

Results of a Study of Cases of Acute Epidemic Poliomyelitis

No.	Age in Years	Duration in Days	Clinical Findings	Necropsy Time after Death	Microscopic Changes
707	4⅔	7	Paralysis of muscles of left side of face, neck and deglutition. Death from respiratory failure	Day of death	Hemorrhage, moderate perivascular and disseminated round-cell infiltration, chiefly in anterior horns and in pia over anterior aspect of cord and medulla
712	4	5	Paralysis of lower extremities, right shoulder and neck. Death from respiratory failure	8 hours	Extreme perivascular and diffuse infiltration chiefly of gray matter of cord and medulla. Perivascular infiltration most marked around vessels extending in horizontally from pia. Moderate infiltration of pia especially around blood vessels and in anterior fissure. Hyperplasia and necrosis of lymphoid follicles in tonsils (Fig. 8). Hemorrhages in mucous membrane of stomach. Small areas of necrosis with round-cell accumulations in liver. Viscera normal otherwise.
714	2	15	Paralysis of legs and shoulders. Death from respiratory failure	4 hours	Cord showed extreme dilatation of vessels; hemorrhages marked degeneration of ganglion cells; slight neurophagocytosis, moderate perivascular and diffuse infiltration with round cells. Slight infiltration in ganglia. No lesions of liver, myocardium, pancreas, kidney and spleen. Hemorrhages and leukocytic infiltration of lung. Marked hyperplasia of lymph follicles in tonsils
720	3	3	Paralysis characteristic of upper spinal type	20 hours	Slight perivascular, moderate infiltration of gray matter of cervical cord with destruction of ganglion cells. No change in viscera
721	9/12	6	Paralysis of meningitic type	Day after death	Typical poliomyelitic changes in cord. No noteworthy changes in viscera other than localized hemorrhages and beginning leukocytic infiltration of lungs
722	3	4	Paralysis of upper spinal type	Day of death	Perivascular infiltration most marked around blood vessels running in from pia, circumscribed areas of round-cell infiltration with destruction of ganglion cells in cord. No changes demonstrable in brain. Areas of necrosis of lymph follicles in tonsils. Liver, pancreas and myocardium normal
723	24	6	Paralysis of right deltoid, right and left quadriceps and psoas, muscles of deglutition and diaphragm	2 hours	Slight round-cell infiltration in pia over cerebrum. Areas of necrosis in lymph follicles in tonsil. Hemorrhages in mesenteric gland. No changes in liver, pancreas, myocardium or kidney
724	1½	3	Paralysis of muscles of neck and left side of face. Death from respiratory failure	12 hours	Brain and cord not saved for microscopic examination
725	1½	4	Paralysis of both legs, back, shoulders and intercostal muscles. Death from respiratory failure	22 hours	Typical poliomyelitic lesions in cord and brain
729	3	9	Paralysis of right side of face, muscles of neck, upper extremities and intercostal muscles. Death from respiratory failure	3 hours	Extreme diffuse round-cell infiltration and numerous hemorrhages in gray matter of cervical cord and medulla. Moderate perivascular infiltration and edema, and slight infiltration of pia, of cerebral cortex and spinal ganglia. Congestion of vessels of liver and kidney. Hyperemia and hyperplasia of lymph follicles in mesenteric gland. Otherwise no noteworthy changes in viscera

Bacteria in Sections	Results in Cultures
Large and small cocci and diplococci in pia of anterior fissure and anterior aspect of cord, in infiltrated areas in anterior horns of cervical cord and medulla (Fig. 2)	Micrococcus and short chained streptococcus varying greatly in size and shape from cord. Diphtheroid-like bacilli in old cultures. Streptococcus from brain and cord after 3 and 15 months in 50% glycerin
Large and small diplococci at least 40 in all, in infiltrated area anterior horns (Fig. 3), ganglia, pia, edematous dura and mesenteric lymph glands. None found in kidneys, liver, lungs, myocardium or sciatic nerve. Many similar organisms found in tonsils (Figs. 9 and 10)	Extremely pleomorphic coccus singly in twos and short chains from cord. Spleen, kidney and liver sterile. Streptococcus and colon bacillus from lumbar cord
Large and small diplococci in areas of hemorrhage and infiltration in anterior horns, in ganglia, in smears from anterior horn and contact films from cerebrum (Fig. 5). Many similar organisms in tonsils and diplococci in hemorrhagic area in lungs. No bacteria in liver, kidney, spleen, myocardium and pancreas	Streptococcus from edematous fluid surrounding dura, from cerebral fluid, brain, lumbar cord and from brain after being dried from 6 to 11 months respectively. Kidney sterile. Streptococcus in pure culture from brain and cord after in 50% glycerin 3, 8, 15, and 16 months
Diplococci in anterior horn of cervical cord and intervertebral ganglia. No bacteria in liver, lung and pancreas	Short chained diplococcus from cervical cord and brain
A few cocci and diplococci in peripheral zone of round-cell infiltration in anterior horn. Many similar organisms in tonsils. Many diplococci in hemorrhagic areas in lungs. No bacteria in liver and kidney	Short chained streptococcus and large and small coccus from cervical cord and brain. Hemolytic streptococcus and colon bacillus from cord
A few large and medium-sized diplococci in peripheral zone of infiltrated areas in gray matter of medulla and cervical cord. A few cocci and diplococci in hemorrhagic areas in mesentery gland. Enormous numbers of same organism in necrotic lymph follicles of tonsils together with fusiform bacilli in crypts of tonsil. No bacteria in brain, liver, pancreas, myocardium	Streptococcus and large and small coccus forms in pure culture from material obtained with pipet from brain and cord, and streptococcus and a few staphylococci from pieces of cord emulsified in mortar, and from mesenteric gland
No bacteria demonstrable in brain. Large number of large and small cocci and diplococci in necrotic lymph follicles in tonsils. Many large forms breaking into small forms. A few large and small diplococci in hemorrhagic mesenteric gland. No bacteria in liver, pancreas, myocardium or kidney	Pleomorphic streptococcus from ventricular fluid, cerebrum, medulla and cervical cord, and diphtheroid-like bacillus from brain
	Large and small streptococcus from brain, pons, and cervical cord and gram-negative bacillus resembling colon bacillus from brain
A few diplococci in cord. No bacteria in myocardium	Medium sized streptococcus and large and small diplococcus from cerebrum, pons and cervical cord
Large and small diplococci in edematous capsule of spinal ganglia, in pia over anterior aspect of cord, in leukocyte in anterior horn, in pons, in edematous areas surrounding infiltrated blood vessels, in mesenteric gland and in contact films from cerebrum (Fig. 6). No bacteria in sections of brain, liver, heart muscle, or kidney. Diplococci in areas of infiltration in sections of cord after in glycerin for 15 months. Many organisms exactly the same in necrotic follicles of tonsils	Pleomorphic streptococcus from blood, spinal fluid, cerebrum, pons, cervical cord, and mesenteric gland. No bacteria in kidney, liver or spleen. Colon bacillus from lumbar cord and mesenteric lymph gland. Streptococcus and large coccus forms from brain and cord after in 50% glycerin for 15 months

TABLE 1—*Continued*

RESULTS OF A STUDY OF CASES OF ACUTE EPIDEMIC POLIOMYELITIS

No.	Age in Years	Duration in Days	Clinical Findings	Necropsy Time after Death	Microscopic Changes
734	7/12	6	Typical ascending paralysis	Day after death	Marked hemorrhages and diffuse infiltration in anterior horns of cervical cord. No changes in brain
745	5½	5	Paralysis of muscles of deglutition and left side of face. Death from respiratory failure	15 hours	Moderate perivascular and slight diffuse infiltration and numerous hemorrhages in gray matter of medulla and cervical cord. Hemorrhages in mesenteric gland. Small areas of necrosis in liver. No changes in lung, pancreas, myocardium and kidney
778	2	15	Paralysis of extremities, muscles of deglutition. Death—respiratory failure	2 hours	Moderate perivascular and circumscribed areas of round-cell infiltration in anterior horns and pia of cord. Marked degeneration and phagocytosis of ganglion cells
779	2	3	Brought to hospital in moribund condition. Death from respiratory failure	20 hours	Moderate perivascular and diffuse infiltration in gray matter of cord and pia. Many hemorrhages chiefly in anterior horns. Many of the infiltrating cells are polymorphonuclear leukocytes
938	12	3	Paralysis of muscles of deglutition, neck and arms. Death from respiratory failure	1½ hours	Extreme diffuse and perivascular infiltration in anterior horn of cord, in medulla and pons; slight in brain. Hemorrhage and leukocytes in alveoli of lung. Hemorrhage in mesenteric lymph gland. Small areas of round-cell infiltration in liver. Localized areas of leukocytic infiltration and hemorrhage in lung. No lesions of other viscera
943	2	1	Paralysis of left side of face, muscles of left leg and thorax. Death from respiratory failure	3 hours	Extreme round-cell infiltration, diffuse and perivascular in anterior horns of cord, moderate of pia and brain. Many of infiltrating cells are polymorphonuclear leukocytes. Extreme hyperemia and hemorrhage in mesenteric gland. Focal necrosis of liver with round-cell infiltration. Leukocytic infiltration in lung. No changes in myocardium, kidney or spleen
948	11	4	Paralysis of right side of face, muscles of neck and thorax. Death from respiratory failure	5 hours	Marked round-cell infiltration of gray matter of cord; perivascular infiltration and areas of hemorrhage. Areas of necrosis in lymph follicles of tonsil. Hemorrhage and hyperplasia of mesenteric lymph glands. Focal necrosis and focal round-cell accumulations in liver. No change in kidney or myocardium
949	17	3	Paralysis of legs, muscles of abdomen, back, upper extremities, of deglutition and diaphragm. Death from respiratory failure	½ hour	Extreme perivascular and diffuse infiltration with round cells in gray matter of medulla. Slight in pia and brain. Areas of hemorrhage and ganglion cell destruction. Areas of necrosis of lymph follicles in tonsils
960	16	3	Paralysis of muscles of deglutition. Death from respiratory failure	8 hours	Necrosis in lymphoid follicles of tonsils and adenoids. Hemorrhage of mucous membrane of stomach, perigastric and mesenteric lymph glands

Bacteria in Sections	Results in Cultures
A few diplococci in areas of hemorrhage in cervical cord. No bacteria in brain	Streptococcus and large diplococcus from brain and cord after in 50% glycerin for 15 months
Large and small diplococci in hemorrhagic areas of medulla. Small diplococci and chain of two in sections of anterior horns in smears from emulsions of cord (Fig. 7b) and in smears from cord after in glycerin for 15 months. Many similar diplococci in necrotic areas in lymph follicles of tonsils	Pleomorphic streptococcus from cerebrum, pons and cervical cord. Streptococcus and large coccus forms from brain and cord after in 50% glycerin for 2 and 15 months, respectively
A few large and small diplococci in pia and infiltrated areas in cord	
A few medium and exceedingly small diplococci in pia and in edematous areas surrounding perivascular infiltrated areas (Fig. 7a)	
Large and small diplococci in edematous areas around infiltrated blood vessels and infiltrated areas in medulla and lumbar cord, and in mesenteric gland. Very many similar organisms in necrotic areas in lymph follicles of tonsils. No bacteria in stomach, kidney, liver or spleen. A few diplococci in pneumonic areas in lung	Streptococcus and coccus forms from brain, pons, medulla, cervical and lumbar cord, and mesenteric gland. Colon bacillus from pons, lumbar cord, mesenteric gland. Kidney and liver sterile
Very large and medium coccus and diplococcus forms in medulla, pia over cerebrum. Many similar organisms in areas of necrosis in follicles of tonsils. A few in hemorrhagic areas in stomach. No bacteria in kidney, myocardium, pancreas, intestine or lung	Streptococcus and large coccus forms from brain, pons, lumbar cord and mesenteric lymph gland. Later also showed colon bacillus. Liver and spleen sterile. Large diplococcus in short chains and tetrads from brain and cord after in 50% glycerin for 3½ and 4½ months
A few diplococci in infiltrated area of anterior horn of cervical cord. Many similar organisms in tonsils. No bacteria in brain, myocardium, lungs, peribronchial lymph gland, mesenteric lymph gland or Peyer's patches	Pleomorphic streptococcus from brain. Streptococcus and large coccus forms from brain and cord after in 50% glycerin for 3½ and 4½ months
Diplococci and chain of two diplococci in pia and anterior horn and in smears from beneath dura of cord after preservation in glycerin (Fig. 7c). Large number of coccus forms singly in twos and short chains in lymph follicles of tonsils	Large and small coccus forms sometimes in short chains from brain, medulla and cervical cord. Streptococcus and large coccus forms from brain and cord after in 50% glycerin for 3½ and 4½ months
Enormous numbers of large medium sized and very small diplococci in lymphoid follicles of tonsils, adenoids and in lymph channels between muscle fibers outside of tonsil. Moderate number in hemorrhagic areas in mucous membrane of stomach and a few in mesenteric glands and lymphoid follicles of Peyer's patches	

"It appears to us that the small, filtrable organism which has been generally accepted as the cause of poliomyelitis may be the form which this streptococcus tends to take under anaerobic conditions in the central nervous system and in suitable culture mediums, while the larger and more typically streptococcic form, which investigators have considered contaminations, may be the identical organism grown larger under suitable conditions."

RESULT OF A STUDY OF CASES WHICH CAME TO NECROPSY

In Table 1 is given a summary of the clinical, pathologic, and bacteriologic findings of 19 cases of acute poliomyelitis in which necropsies were done.* The ages of the patients ranged from 7 months to 24 years; only 5 were more than 6 years. The duration of symptoms was from 1-15 days. The extent of flaccid paralysis varied greatly in the different cases. Nearly all these patients died of paralysis of the muscles of respiration. Bronchopneumonia was found in four; a terminal rise in temperature suggesting antemortem infection occurred in eight of the cases, but was absent in the others. The necropsies were all done within 24 hours after death; one in ½ hour, one in 1½ hours, two within 2 hours, and seven within 12 hours.

The gross and microscopic findings in all were typical of acute poliomyelitis. The viscera usually showed no gross changes other than congestion and cloudy swelling. The mesenteric lymph glands were swollen in most of the cases. Peyer's patches were swollen, and the lymphoid follicles in the intestines and mesenteric glands were edematous and hemorrhagic in cases in which there were marked gastro-intestinal symptoms. Localized hemorrhages in the mucous membrane of the stomach had also occurred in a number of the cases. As a routine, portions of tissues were immediately placed in 10% formalin and in some instances in Kaiserling's fluid, for microscopic study. They were embedded in paraffin, and sections from 5-10 microns thick were stained with hematoxylin and eosin, methylene-blue and eosin, and with eosin and polychrome methylene blue, for microscopic study.

The microscopic changes in the brain and cord were typical of poliomyelitis in all the 17 cases examined. The pia in all the cases showed some round-cell infiltration and in a few of the cases this was quite prominent, especially in the anterior fissure and over the anterior aspect

* The material from nine cases was obtained through the courtesy of Drs. Anna Williams and H. L. Abramson of the Health Department of New York City.

of the cord (Figs. 1 and 3). Perivascular infiltration was variable but usually marked and consisted of small, round, deeply staining cells (Figs. 1 and 3). Infiltration was often very noticeable around the vessels which extend in horizontally from the pia in the anterior aspect of the cord (Fig. 3). Round-cell infiltration occurred in gray and white matter and in the ganglia, but was especially marked in the anterior horns. This was usually diffuse over wide areas, but small circumscribed areas were found adjacent to, and at times widely separated from, areas of diffuse infiltration. The cells were larger and stained less deeply than those around the blood vessels and often contained elongated nuclei. Neurophagocytosis was prominent in some of the cases. Only moderate infiltration was found in the posterior root ganglia. Polymorphonuclear cells were found in infiltrated areas in all but the 2 patients (Cases 714 and 778) who died 15 days after the onset of paralysis. In the others they were usually few in number, although in some running a short course they were quite numerous. In three cases fully one-third of the cells in some areas were polymorphonuclear leukocytes (Cases 779, 938, and 943). This finding confirms the observations made by Robertson and Blanton, namely, that polymorphonuclear cells appear to be especially prominent in the patients who die early, and are prone to be absent or few in number in those who die relatively late.

Sections of mesenteric lymph glands, Peyer's patches, and, less often, of the spleen, showed hyperemia, hemorrhages, and, in some, hyperplasia of the lymph follicles. The liver in 4 cases showed the small areas of necrosis with the accumulation of round cells described by Peabody, Draper and Dochez. The lungs in 4 cases showed hemorrhage and leukocytic infiltration. In the rest of the organs there were no noteworthy changes aside from a certain degree of congestion of the vessels and granular swelling of parenchymatous cells.

THE DEMONSTRATION OF BACTERIA IN THE CENTRAL NERVOUS SYSTEM

Search for bacteria was made in the brain and cord and other organs in 14 cases, and in the brain and cord in 3 additional cases. The material studied consisted of films made directly from the brain surface, from material aspirated with pipets from the anterior horns and from emulsions in salt solution of brain and cord and mesenteric glands, and of sections of brain, cord, ganglia and other viscera. The

latter were usually fixed in 10% formalin and in some instances in Zenker's or Kaiserling's solution. Sections were stained for bacteria by Gram-Weigert's or Giemsa's method, and with eosin and polychrome methylene blue. The films were usually stained by Giemsa's method. In the Gram-Weigert method decolorization was carried to the point where the cells showed a pale-blue tinge. If decolorization is carried farther than this the micro-organisms may lose the stain and the areas showing lesions cannot be made out clearly. Artefacts such as precipitated stain, cross-sections of nerve fibers and chromatin granules have been eliminated.

Coccus and diplococcus forms in chains of two or three and in small masses were found quite readily in sections of the central nervous system which showed typical poliomyelitic changes. They were demonstrated in all but 1 of the 17 cases. In the negative case, material from the brain only was available for study. Both film preparations and sections showed unmistakable organisms (Figs. 2, 4, 5, 6 and 7). They were found in contact films from the cerebrum, in films from the pipetted material from edematous anterior horns and in emulsions of the cord. The identical organisms were found in sections, in edematous areas in the dura, in the infiltrated pia, in the anterior fissure and surrounding the vessels in the pia along the anterior aspect of the cord, in edematous dura surrounding the intervertebral ganglia, in infiltrated ganglia, in areas of perivascular and diffuse infiltration in the gray matter of the cord, in walls of blood vessels and in dilated capillaries in edematous areas in the anterior horns, and in infiltrated areas in the brain. In 2 cases they were found in the cord, but not in the brain. These findings are in accord with those recently reported by Hektoen, Mathers and Jackson.

The same organism was found in small numbers in sections of mesenteric lymph glands in 6 cases. Diplococci of medium size were found in infiltrated areas in the lungs in 4 cases, and in hemorrhagic areas in the mucous membrane of the stomach, and in Peyer's patches in 2 cases.

No bacteria were demonstrable in sections of the other organs or in areas of brain and cord which were free from lesions. Bacilli or other microbic forms have not been found by us.

. The micro-organisms were usually extracellular, but occasionally were found within cells. They varied greatly in size, shape and grouping in different locations in the same case, but were much alike

in the different cases and occurred in widely separated regions. The variations in size were noted even among the minute forms and when arranged in short chains (Fig. 5b). The very small forms often occurred in groups commonly containing 4 or 8 and sometimes 16 minute cocci, usually in pairs, suggesting the rapid breaking up of large coccus forms into the small forms (Figs. 2c, 6b, and 7a). The very large coccus or diplococcus forms usually occurred along blood vessels or capillaries.

CULTURES FROM THE TISSUES

The cultures were made essentially according to the methods used by one of us in a study of the bacteriology of tissues from various diseases,[15] and that used by Flexner and Noguchi. To obtain the initial growth three mediums were regularly used: (1) Ascites-dextrose broth and dextrose broth in tall (12 cm.) and short (8 cm.) tubes and bottles with or without sterile tissue; (2) ascites-dextrose-agar and dextrose-agar, usually without tissue, and (3) tall tubes of unheated ascitic fluid plus sterile tissue and oil. The dextrose broth and agar were prepared with Witte's peptone and Liebig's extract of beef, titrated to 0.6-0.8 acid to phenolphthalein. The broth contained 0.2% dextrose, the agar 1% dextrose and 1.5% agar. The ascites fluid was of high specific gravity, was free from bile, and was previously proved sterile by making aerobic and anaerobic cultures from the sediment of lots of approximately 200 c c each. Pieces of brain, medulla, cervical cord, etc., were removed with sterile precautions and emulsified in a sterile air chamber or fragments planted directly. In some instances pipetted material from the anterior horns or the brain, after searing the surface, were also cultured. Usually a large number of tubes were inoculated with varying amounts of emulsions. Approximately 1 c c of a 10% emulsion in normal salt solution per 10 c.c. of medium gave the best results. Both too little and too much emulsion per cubic centimeter might lead to negative results. The cultures were incubated at 35 C. Only a few were put in the anaerobic jar, as it was thought desirable to make frequent smears of all cultures which grew.

Cultures were made from different parts of the central nervous system, usually of spinal or ventricular fluid, brain, pons, medulla, edematous dura, ganglia, and from different levels of the cord. A gram-staining coccus varying greatly in size, shape and grouping, but

usually essentially streptococcal in character, has been isolated in all of 15 cases. In eight of the strains, chain formation was relatively marked and in seven it was relatively slight. In most instances the emulsions of all the specimens cultured yielded this organism, but not in all the tubes inoculated. The spinal fluid cultures remained sterile except in two instances, and these showed the characteristic streptococcus. Colon bacilli were obtained usually in only one or more fragments or tubes from 6 cases, hemolytic streptococcus in one instance, diphtheroid bacilli in two, and in a few instances Bacillus subtilis and Staphylococcus albus.

Cultures of emulsions of brain and cord after preservation in 50% glycerol in the ice-chest for from 3-16 months have yielded microorganisms identical to the one isolated from the fresh tissues in all but two of 16 sets of cultures which were made from material from 8 cases.

Cultures from mesenteric lymph glands were made in 4 cases. All yielded the characteristic micro-organism and in 2, colon bacilli were obtained in addition. The streptococcus isolated constantly from the central nervous system was not found in a single instance in other organs cultured in 5 cases. This result does not bear out the opinion expressed by Kolmer and Freeze, that this organism was probably present in these organs, but adds materially to the significance of their complement-fixation tests with antigens prepared from these strains.

The cultures in the short tubes of dextrose broth and bottles showed usually a diffuse granular turbidity in from 24-48 hours. Those inoculated with emulsions grew more often than those inoculated with fragments. Smears at this time showed cocci and diplococci often in short chains and occasionally in long chains of uniform size or, more often, of very irregular size. Subcultures on blood-agar plates at this time gave a growth of small, dry, green-producing colonies which might be surrounded by a narrow, hazy zone of hemolysis, especially after 48-72 hours' incubation. In old cultures in the short tubes of ascites-dextrose broth and, with few exceptions, in tall tubes containing this medium, the organisms become gram-negative as they die, but do not grow to small size (Figs. 12a and 13a). In shake cultures of the emulsion in short tubes of ascites-dextrose-agar small, discrete, grayish colonies, always in small numbers, appeared in the deeper portion of the medium, never above 1.5 cm. from the top, usually on the 2nd or 3rd day. The cultures in ascites fluid plus tissue

and oil grew better when inoculated with fragments of nervous tissue than with emulsions. Growth in these tubes rarely became evident before the 3rd, more often after the 5th to the 10th day. Growth was manifested by the appearance of a diffuse cloud in the lower portion of the tubes which gradually increased and extended to the upper portion of the medium, but it rarely extended above about 3 cm. from the top. As this occurred the cloud in the lower portion became granular, a sediment appeared, and small colonies collected along the sides of the tubes. Tubes inoculated with emulsions showed similar but less constant results.

Smears from the bottom of the tubes of ascites-tissue fluid in which clouding was perceptible as early as the 3rd or 5th day, showed coccus and diplococcus forms as large as in the aerobic cultures together with numerous smaller forms. After a week or 10 days these cultures and those in which clouding first appeared in from 7-10 days showed enormous numbers of extremely small cocci, diplococci and short chains, often occurring in clumps. These small forms lost the Gram stain quite readily, stained purplish with Giemsa, were fully as small as, and appeared in every way to be identical with, the "globoid" organism described by Flexner and Noguchi. The organism isolated in ascites-dextrose broth remains an ordinary-sized diplococcus when grown aerobically. Transfers of aerobic cultures even after repeated platings to ascites fluid plus tissue and oil always resulted in growth of the small forms. Filtrates through Berkefeld N filters of these cultures have repeatedly yielded the organisms characteristic of these mediums. Cultures which showed only globoid bodies and which would not grow in aerobic broth or on plates, were grown back to large lanceolate diplococci and cocci without difficulty if they were not more than 3 or 4 weeks old. Those which had been under anaerobic conditions for a longer time could be grown back to large size, but with greater difficulty. Transfer of rather large amounts of the culture to short tubes of ascites-dextrose broth plus tissue was a good method to bring this about. Another good method to obtain the large aerobic forms was to stab tall tubes of ascites-dextrose-agar plus tissue and to make subcultures from the highest point of growth.

Generally speaking, there was a marked tendency of the small organisms in the anaerobic cultures to grow large when placed under aerobic conditions. It has frequently happened that shake-cultures from these in ascites-tissue-agar showed exclusively small organisms

in colonies which grew only in the lower portions of the tube and which, on being transferred to ascites-dextrose broth showed large organisms varying greatly in size and shape, some resembling streptococci, others staphylococci and diphtheroid bacilli. These at first were considered contaminations, but since the different forms occurred in the same chain, and developed in strains after repeated platings, fishing single colonies each time, under conditions in which entrance of extraneous organisms was carefully excluded, they were held to be variants or mutations forms. Moreover, by following the methods of cultivation described, the details of which are to be published in another paper, two strains of pure cultures of the "globoid" organism obtained through the kindness of Dr. Noguchi have been made to grow aerobically on repeated occasions. This has been done with cultures made from single colonies and in media where the possibility of contamination from the tissue and ascites fluid was excluded. In the aerobic condition the size, morphology and character of growth resemble closely the aerobic form as we isolate it constantly from poliomyelitic tissue.

At first it was thought that the small globoid organism in ascites-tissue fluid (Fig. 13b), as described by Flexner and Noguchi, was characteristic of this medium, but by making smears from the bottom of the tubes daily after inoculation, it was found that the minute forms observed after some days of incubation and after evidence of growth was apparent, were the result of the breaking down of larger forms present before clouding occurred. Mathers, and Nuzum and Herzog have reported similar cultural results in cases of poliomyelitis, but appear to have overlooked this point. Kolmer, Brown and Freeze,[7] who also report similar findings, state that "Films of the aerobic and young anaerobic cultures showed a gram-positive coccus usually arranged in diplococcus formation in chains of four or five pairs and in clumps. Films of older anaerobic cultures (fourteen days or more) showed that the majority of these cocci had become smaller and many were easily decolorized by alcohol in the Gram stain."

A detailed study of this point was made by Rosenow and Towne with identical strains isolated from the brain and cord of monkeys paralyzed with virus in the usual way. By studying the method of growth in accordance with oxygen-tension the mechanism by which the large forms become small and the small forms large has been determined. They grow small in anaerobic cultures of ascites fluid plus

tissue and oil and large in aerobic cultures of ascites-dextrose broth. Rosenow and Towne have already suggested that "among many factors concerned in causing these changes in size, surface tension may be of importance. If there are so many large diplococci that their metabolic requirements cannot be met by the medium, division which gives more surface for the same sized body might allow a certain number of the resulting smaller forms to survive."

The growth of the small organisms to large size under aerobic cultivation in ascites-dextrose broth where oxygen and available nutrient material are abundant would seem to be the result of the same factors working in the opposite direction. It appears quite as if the alterations in size occur according as certain metabolic or oxygen requirements need to be maintained. The occurrence of large, medium-sized and small forms in the tissues as shown in this paper would appear to be due to the same causes. The fact that large forms are prone to occur along blood vessels in the tissues where available oxygen would appear to be relatively high is thus explained, and hence these large forms should not be considered as being contaminations or as distinct from the small forms. A certain degree of adaptation or alteration in these requirements, however, appears to take place, because the organism, when kept under strict anaerobic conditions for a long time resists aerobic cultivation, a power which it can again be made to acquire gradually. The fact that unusually large forms are prone to develop when growth does occur (Figs. 13c and 13d) after anaerobic cultivation, and when first isolated from the tissues (Fig. 11), a property which is lost on continued aerobic cultivation, is also in harmony with this idea.

CHARACTERISTICS OF THE MICRO-ORGANISM FROM POLIOMYELITIS

In Table 2 is given a summary of the morphologic character, of growth on blood-agar, solubility in bile, etc., and the fermentative powers of strains isolated from tonsils and central nervous system of 35 cases of poliomyelitis. Altogether, the fermentative powers of strains from 49 cases have been studied, but the other tests were not made at the same time and hence are not included in the table. Twenty of the strains summarized in the table were isolated from the tonsils or the throat, 3 from stool and 17 from brain or cord. The culture generation is indicated by the figure following the point and

TABLE 2

Characteristics of Micro-Organisms Isolated from Acute Epidemic Poliomyelitis

Strain	Source	Character of Growth on Blood-Agar Plate	Ascites Dextrose Broth		
			Turbidity	Sediment	Morphology
686.1[2]	Tonsil	Small dry colonies with green halo. No hemolysis	Diffuse granular cloud	Granular	Irregular short chained streptococcus, some very small. Large and small forms often in same chain
686.2[2]	Tonsil	Small dry colonies with green halo. No hemolysis	Diffuse granular cloud	Granular	Irregular short chained streptococcus, some very small. Large and small forms often in same chain
686.2	Tonsil	Small colonies with green halo. No hemolysis	Diffuse granular cloud	Flocculent	Irregular short chained streptococcus, some very small. Large and small forms often in same chain
698.3	Tonsil	Grayish green indifferent colonies	Diffuse granular cloud	....	Irregular diplococci in short chains
699.7	Tonsil	Small dry colonies with green halo	Diffuse cloud	Flocculent	Irregular diplococci in short chains
700.3	Tonsil	Small colonies with green halo	Diffuse cloud	....	Short chained pneumococcus-like streptococcus
701.4	Tonsil	Grayish indifferent colonies	Diffuse cloud	....	Short chained pneumococcus-like streptococcus
704.7	Tonsil	Moist colonies with green zone	Slight granular cloud	Flocculent	Irregular diplococci often in short chains
707.10	Cord	Small dry colonies with narrow green zone	Slight granular cloud	Flocculent	Irregular micrococcus type. Short chains and tetrads
707.4	Cord after in glycerin 16 months	Small dry colonies. Faint green zone in first two cultures then slightly hemolyzing zone	Diffuse granular cloud	Flocculent	Irregular short chained streptococcus
708.10	Tonsil	Medium dry colonies............	Diffuse cloud	Flocculent	Medium size, decidedly irregular diplococcus in short chains
708.15	Tonsil	Small dry colonies..............	Diffuse cloud	Flocculent	Medium size, regular round diplococcus in short chains
711.3	Tonsil	Small dry colonies with green halo	Diffuse cloud	Flocculetn	Pneumococcus-like diplococcus in short chains
714.11	Brain	Small dry colonies with green halo	Diffuse cloud	Granular	Round short chained streptococcus
714.18	Brain	Small dry colonies with green halo	Diffuse cloud	Granlar	Irregular streptococcus. Involution forms and cocci dividing transversely
714.27	Tonsil	Moist colonies with green halo	Diffuse cloud	Flocculent	Small round regular medium chained streptococcus
714.22	Brain	Moist colonies with green halo	....	....	....
714.47	Brain	Large moist colonies with green halo	Diffuse cloud	Flocculent	Round medium short chained streptococcus
714.3	Brain after in glycerin 16 months	Small dry colonies with green in first culture, slightly hemolyzing colonies in third culture	Diffuse cloud ular cloud	Flocculent	Round short chained streptococcus
721.5	Brain	Small dry colonies with green halo and larger more opaque grayish colonies	Diffuse cloud	Granular	Short chain streptococcus, varying size and shape, coccus forms and chains from single colonies
722.4	Brain	Small dry colonies with green green halo	Diffuse cloud	Granular	Short chain streptococcus of varying size and shape, coccus forms and chains from single colonies
722.3.12	Brain	Translucent colonies	Diffuse cloud	Granular	Short chain streptococcus of varying size and shape, coccus forms and chains from single colonies
722.12	Brain	Small colonies with green halo	Diffuse cloud	Granular	Short chain streptococcus of varying size and shape, coccus forms and chains from single colonies

TABLE 2.—Continued

CHARACTERISTICS OF MICRO-ORGANISMS ISOLATED FROM ACUTE EPIDEMIC POLIOMYELITIS

Litmus Milk			Gelatin	Precipitation in Aseites Dextrose Agar	Solubility in Bile	Acid Production in Broth								
Acidity	Decolorization	Coagulation				Plain	Dextrose	Lactose	Maltose	Saccharose	Raffinose	Mannite	Salicin	Inulin
+	0	0	0	+	0	0	+	+	+	+	No growth		..	0
+	0	. 0	..	..	0	0	+	+	+	+	+	No growth	+	0
+	0	+	..	..	..	0	+	+	+	+	+	No growth		0
+	0	0	0	..	..	0	+	+	+	+	+	0	+	+
+	0	0	0	+	0	0	+	+	+	0	0	0	+	0
..	..	..	..	..	..	0	+	+	+	+	+	No growth	+	+
..	..	..	..	..	..	0	+	+	+	+	+	0	+	+
+	0	0	0	+	0	0	+	+	+	+	0	0	0	0
+	0	0	0	+	..	0	+	+	+	+	+	0	+	0
+	+	+	0	+	0	0	+	+	0	+	+	0	0	+
+	0	+	0	0	..	0	+	+	+	+	+	0	0	0
No growth			0	0	+	0	+	+	+	+	0	0	0	0
+	0	+	0	0	0	0	+	+	+	+	0	0	0	+
+	0	+	0	+	0	0	+	+	+	+	+	0	0	0
+	+	+	0	0	0	0	+	+	+	+	+	0	0	0
+	0	0	0	+	0	0	+	+	+	+	0	0	0	0
..	..	..	..	..	..	0	+	+	+	+	0	0	0	0
+	0	0	0	+	0	0	+	+	+	+	0	0	0	0
+	+	+	Liquified	+	0	+	+	+	+	+	0	+	+	0
+	+	0	0	+	0	0	+	+	+	+	+	+	+	0
..	..	..	..	..	..	0	+	+	+	+	+	0	+	-
..	..	..	..	..	..	0	+	+	+	+	No growth		+	+
..	.	..	..	..	..	0	+	+	+	+	+	No growth	+	+

TABLE 2.—*Continued*

CHARACTERISTICS OF MICRO-ORGANISMS ISOLATED FROM ACUTE EPIDEMIC POLIOMYELITIS

Strain	Source	Character of Growth on Blood-Agar Plate	Ascites Dextrose Broth		
			Turbidity	Sediment	Morphology
723.5	Tonsil	Chocolate colored colonies......	Granular cloud	Granular	Irregular diplococcus ....
474.7	Cord	Moderate moist colonies with green halo	Slight cloud	Granular	Irregular short chained streptococcus hemolyzing pneumococcus
725.2	Tonsil	Chocolate colored colonies......	Slight cloud	Granular	Irregular short chained streptococcus resembling pneumococcus
729.10	Cord	Small moist and indifferent colonies	Diffuse cloud	Flocculent	Large oval diplococcus. No chains
729.7	Brain	Large moist indifferent colonies	Diffuse cloud	Flocculent	Medium round diplococcus........
730.10	Tonsil	Small dry green colonies........	Dffiuse cloud	Flocculent	Small round diplococci in clumps and short chains
731.2	Tonsil	Grayish colonies	....	....	Small round diplococci in clumps and short chains
732.8	Tonsil	Small dry colonies with green halo	Granular cloud	Flocculent	Very irregular streptococcus, short and long chains
733.2	Tonsil	Small dry colonies with green halo	Granular cloud	Flocculent	Very irregular streptococcus, short and long chains
734.2	Tonsil	Small dry colonies with green halo	....	....	Very irregular streptococcus, short and long chains
773.2	Stool	Moderate moist colonies with green halo	Diffuse	Slightly flocculent	Large micrococci singly in diplococci and short chains
836	Brain or cord	Small dry colonies with green halo	Diffuse	Granular	Large oval diplococcus..........
837	Brain or cord	Grayish green staphylococcus-like. Some hemolyzing colonies	Flocculent	Flocculent	Micrococcus type ....
838	Brain or cord	Grayish green staphylococcus-like. Some hemolyzing colonies	Flocculent	Flocculent	Micrococcus type ....
839	Brain or cord	Indifferent staphylococcus-like colonies	Diffuse	Granular	Micrococcus type. Quite staphylococcus-like
840	Brain or cord	Small dry colonies; slight hemolysis around colonies	Diffuse	Granular	Large lanceolate diplococcus in clumps and short chains
841	Brain or cord	Small dry colonies; slight hemolysis around colonies	Diffuse	Granular	Large lanceolate diplococcus in clumps and short chains
842	Brain or cord	Small dry colonies with green halo	Diffuse	Granular	Large lanceolate diplococcus in clumps and short chains
899	Pons	Opaque grayish green colonies	Slightly diffuse cloud	Flocculent	Micrococcus type, tetracocci, diplococci and short chains
938.6	Tonsil	Small dry colonies with green halo	Diffuse cloud	Flocculent	Irregular short chained streptococcus
938.7	Pons	Small dry colonies with green halo	Diffuse cloud	Granular	Irregular short chained streptococcus
943.6	Stool	Small dry colonies with green halo	Granular cloud	Granular	Medium short chained, elongated streptococcus
943.6	Tonsil	Small dry colonies with green halo	Granular cloud	Granular	Medium short chained, elongated streptococcus
943.3	Brain	Small dry colonies with green halo	Granular cloud	Granular	Medium short chained, elongated streptococcus
943.3	Lumber cord	Small dry colonies with green halo	Granular cloud	Granular	Medium short chained, elongated streptococcus
948.3	Tonsil	Large opaque colonies with greenish tinge	Diffuse cloud	Flocculent	Irregular diplococci, some very large, others medium size
948.3	Brain	Large opaque colonies with greenish tinge	Diffuse cloud	Flocculent	Irregular diplococci, some very large, others medium size
979.3	Stool	Small dry colonies with green halo	Diffuse cloud	Flocculent	Irregular diplococci, some very large, others medium size

| Litmus Milk | | | Gelatin | Precipitation in Ascites Dextrose Agar | Solubility in Bile | Acid Production in Broth | | | | | | | | |
Acidity	Decolorization	Coagulation				Plain	Dextrose	Lactose	Maltose	Saccharose	Raffinose	Mannite	Salicin	Inulin
..	..	..	..	..	..	0	+	+	+	+	+	0	+	0
0	0	0	0	0	+	0	+	+	+	+	+	+	+	+
..	..	..	..	..	..	0	+	+	+	+	+	0	0	0
+	+	0	0	+	0	0	+	+	+	+	+	+	+	0
+	0	0	0	+	+	0	+	+	+	+	+	+	+	0
+	0	0	0	+	0	0	+	+	+	+	0	0	0	0
..	..	..	..	..	..	0	..	+	+	+	No growth		+	0
+	0	0	0	0	0	No growth	+	+	+	+	0	0	0	0
..	..	..	..	..	..	0	+	+	+	+	+	No growth		+
..	..	..	..	..	..	0	+	+	+	+	0	+	+	0
+	+	0	0	+	0	0	+	+	+	+	No growth	0	+	0
+	0	0	0	+	0	0	+	+	+	+	0	+	+	0
0	0	0	0	+	0	0	+	0	+	+	0	0	0	0
0	0	0	0	+	0	0	+	0	+	+	0	0	0	0
+	0	0	0	+	0	0	+	+	+	+	0	0	0	0
+	+	0	0	+	0	0	+	+	+	+	0	+	+	0
+	+	0	0	+	0	0	+	+	+	+	0	+	+	0
+	+	0	0	+	0	0	+	+	+	+	0	+	+	0
+	0	0	Liquified	+	0	0	+	+	+	+	0	+	+	0
+	0	+	0	0	0	0	+	+	+	+	+	0	0	No growth
+	..	..	0	..	..	0	+	+	+	+	0	0	+	+
..	..	..	0	..	..	0	+	+	+	+	+	0	+	+
..	..	..	0	..	..	0	+	+	+	+	+	0	+	+
..	..	..	0	..	..	No growth	+	+	+	+	+	0	No growth	+
..	..	..	0	..	..	0	+	+	+	+	+	No growth		+
+	0	0	0	..	..	0	+	+	+	+	+	+	+	+
+	0	0	0	..	..	0	+	+	+	+	+	No growth	+	+
+	0	0	0	..	..	0	+	+	+	+	+	0	+	+

the animal passage by the exponent to the right and above the figure indicating the strain or case number. Standard blood-agar made from Liebig's extract of beef and Witte's peptone to which about 0.3 c c per 5 c c of agar of defibrinated human blood was added, was used for the plates. Twenty-nine of these strains produced small, dry colonies with a green halo and sometimes slight hemolysis after from 48-72 hours; 7 produced somewhat more moist colonies with a greenish tinge resembling virulent pneumococci; 8 produced small grayish or chocolate-colored, indifferent colonies, and 7, larger, more opaque, grayish-white colonies resembling staphylococci. On this medium the organims were quite uniform in size and resembled pneumococci but were usually smaller and free from demonstrable capsule. The growth in ascites-dextrose broth or dextrose broth in columns about 7 cm. tall of the strains soon after isolation was relatively slow. Diffuse turbidity or a flocculent growth collecting along the side of the tube was usually noted in from 48-72 hours unless the inoculation was heavy, when marked growth occurred in 24 hours. After cultivation for a time the growth became more rapid. Most of the strains on isolation showed a preference for relative anaerobic conditions. It was the rule that growth in these tubes began at the bottom and was then forced slowly to the top as oxygen-tension was lowered and as the bacteria multiplied. Frequently the top 0.5 to 2 cm. were perfectly clear, especially in large amounts of medium in bottles, when the deeper layers showed dense growth. This clear layer was not observed in the tubes to which a thick layer of oil was added. The preference for relative anaerobic conditions was usually lost after a number of aerobic transplants, but it was preserved and at times was regained on anaerobic cultivation in ascites-tissue fluid.

The morphology in ascites-dextrose broth, as given in the table, varied considerably in the different strains and according to the time of and method of previous cultivation. In all but 7 strains the form was distinctly streptococcal, smears showing short chains of diplococci resembling pneumococci, a smaller number of medium-sized cocci in pairs of varying size and occasional large and very small diplococcus and coccus forms (Figs. 11, 12 and 13). Most of the strains had been grown aerobically for some time and had lost much of the tendency to grow with marked variations in shape and size. They approached ordinary-sized diplococci or short-chained streptococci.

Practically all the strains acidified, but only a small number decolorized or coagulated litmus milk. Only one strain liquefied gelatin.

Tweny of 27 strains produced clouding of ascites-dextrose agar; 3 of 26 were dissolved by bile. All produced acid in dextrose, maltose and saccharose and all but 2 in lactose. Twenty of 45 strains produced acid in raffinose, 11 of 39 in mannite, 28 of 44 in salicin, and 17 of 48 in inulin. In 3 cases the strains from tonsil and brain or cord, in 1 case from tonsil, stool, brain and cord, and in 5 cases the strain before and after from 1-4 animal passages showed practically identical fermentative powers. This was true in some instances in which a given strain was passed through different species of animals, and hence the strains isolated from the animals were not contaminants. The power to produce acid in inulin by these strains, as is true with pneumococci from lobar pneumonia, tends to be lost on prolonged cultivation, especially on blood-agar. Thus, 6 strains produced acid soon after isolation but not later in repeated tests. Of the total number of 141 cultures in which the fermentation tests were made, 69 were made soon after isolation, 33 fermenting inulin, while of 72 in which the tests were made from 3-15 months after isolation, only 7 fermented inulin. All the strains tested from the Davenport epidemic fermented inulin and all these tests were made soon after isolation. None of the Philadelphia strains* (Cases 836 to 899) fermented inulin. All the tests were made after the strains had passed through numerous cultures.

It is noteworthy that none of the 3 strains isolated from the stool in 3 cases fermented mannite, and hence they should not be regarded as Streptococcus fecalis. The morphology and character of growth resembled closely the strains isolated from the tonsil and nervous system. Moreover they had common agglutinative properties.† Gastro-intestinal symptoms were pronounced in each of these patients. The remaining 7 strains showed large diplococci singly, in masses and rarely in short chains. Mathers, and Nuzum and Herzog, and Kolmer, Brown and Freese also report the isolation of these 2 types of strains. The former consider them as the same organism, the latter as distinct. These strains produced larger and more opaque colonies than the streptococci. It has frequently happened that both these types have been isolated from the same case, both varieties have developed from widely separated single colonies and from pure cultures of the streptococcus after repeated platings. The diplococcus or micrococcus type of growth is particularly prone to develop from the streptococcus when

* We are indebted to Dr. B. Lucke and Dr. M. Solis-Cohen for these strains.
† For the agglutinating power of these strains and their infective power, reference is made to other papers in this series.

pure cultures of the latter in old anaerobic cultures in ascites fluid plus tissue and oil or ascites-tissue-agar stabs are transferred to dextrose broth and then plated, or to blood-agar plates directly. Observations made in Case 721 are similar to many others that have been made:

Sept. 5, 1916.—Smears from single colonies in ascites-dextrose-agar and dextrose-agar shake-cultures showed chains and pairs, some evidently streptococcus, others resembling staphylococci. Blood-agar plates showed pure culture of small, dry, green colonies. One of these colonies was stabbed and inoculated into the agar. Sept. 9.—Blood-agar plate from stab made from above colony in dextrose-agar again showed pure culture of small, dry, green colonies. Single colonies from blood-agar plate to blood-agar plate were now transferred daily for 6 days. The power to produce green colonies gradually diminished but otherwise pure, fine, dry colonies were obtained in each plating. A single colony was again plated and inoculated into a tall tube of dextrose broth. The plate-to-plate culture again showed fine dry colonies, while platings from the broth the following day yielded countless small colonies a little more moist than before, showing no green or hemolysis and among them larger, more opaque staphylococcus-like colonies with large diplococci and tetracocci. The latter resembled exactly those obtained with the streptococcus from dextrose-broth cultures of the emulsion of the brain and cord in this case, and until this observation was made were considered contaminations. A close distinction, therefore, between these two forms should not be made.

Case 695.—N. L., a boy, aged 4 years. (Patient of Dr. G. T. Joyce, Rochester, Minn.)

July 28, 1916.—The illness began with cough, headache, high fever and marked constipation. Flaccid paralysis of the right leg developed on the second day, of the right arm and left leg on the third, and marked weakness of the left arm and difficulty in breathing on the fourth. Much mucopurulent material was found in the nasopharynx. The throat was moderately red. The tonsils were small and appeared quite normal on the surface but pus was expressed. The spinal fluid was under pressure, and clear. The patient recovered with marked persistent residual paralysis. This child, together with his older brother, aged 6 years, had an attack of bronchitis with fever about 10 days previous to the onset of the paralytic attack. Both showed a recurrence of the bronchial infection, but the older brother recovered without developing paralysis.

Aug. 3.—Blood-agar plate of pus from tonsil showed enormous numbers of fine, dry colonies with a green halo, a few fine hemolyzing colonies, and a few larger, more opaque colonies.

Aug. 4.—Blood-agar plate of pus obtained from tonsil the second time showed a similar result. Ascites-dextrose-broth cultures from pus and from single colonies showed streptococci varying greatly in size and shape. In some instances very large budding forms were seen in chains containing typical diplococci. Large coccuslike forms in chains were seen to break into two and four smaller forms (Fig. 11). Cultures from the tonsil on three subsequent dates showed a gradual diminution in the number of fine, dry, hemolyzing colonies.

Case 707.—W. M., a boy, aged 4 years, was admitted to the New York Hospital Aug. 18, 1916. The disease began 6 days previously with high fever and vomiting, after which the child was better for 3 days. He grew nervous and

restless and again vomited a number of times 3 days previous to admission to hospital, developing weakness and asymmetry of the left side of the face, and difficulty in swallowing. The patient was inert and stuporous, with rigidity of neck and back. The respirations were rapid and performed mostly with the accessory muscles. He cried out weakly and was unable to take food. A spinal puncture was made; the fluid was not under increased pressure and 15 c c were withdrawn. There were 40 cells per cubic millimeter.

Aug. 19.—The child died of respiratory failure. There was a rise of temperature to 103 F. immediately before death. This patient had had tonsils and adenoids removed several months previous to the attack of poliomyelitis. A partial necropsy was obtainable. There were edema and hemorrhages of the dura, marked congestion of the vessels of the pia of the cord associated with marked edema, a thin fibrinous film covering the pia, and a markedly increased amount of clear spinal fluid. Cross-sections of the cord showed extreme hyperemia of the vessels, hemorrhages and edema, especially of the gray matter of the anterior horns. The spinal ganglia appeared edematous. One and one-half centimeters of dorsal cord were removed in a sterile manner, emulsified in a sterile air chamber and cultures made immediately in dextrose agar, and ascites-dextrose agar, in ascites, tissue fluid covered with paraffin-oil, and in plain and dextrose broth with and without sterile ascites fluid.

Aug. 21.—Ascites-dextrose broth showed granular turbidity, and smears showed large numbers of large and small gram-positive and gram-negative diplococci and short chains. The larger organisms resembled pneumococci. Films from one colony 1.5 cm. from the top of the ascites-dextrose-agar shake culture showed large lanceolate-shaped and smaller, more rounded diplococci. Sometimes both varieties occurred in the same chain. No other colonies were detected in the shake cultures. Ascites-fluid culture showed no increase in turbidity. Ascites-dextrose-broth culture showed diffuse granular cloud and stained films, short-chained, irregular streptococcus.

Aug. 23.—Blood-agar plate cultures of each of these showed pure growth of fine, dry, nonadherent colonies of streptococci surrounded by a faint green zone. The blood-agar plates which had been inoculated with the single colony in dextrose-agar were used to inoculate ascites-dextrose broth, and tall tubes of ascites-plain-tissue broth and ascites-tissue fluid to which a layer of oil was added.

Aug. 24.—The dextrose-broth culture showed diffuse granular cloud with flocculent sediment and many gram-staining diplococci of irregular size. The ascites-plain-tissue broth and ascites-tissue fluid showed no turbidity.

Aug. 26.—The latter two cultures showed a dense collection of fine colonies in the bottom, appearing as a granular growth loosely adherent to the side of the tubes. These became gradually fewer in number and smaller in size up to 3.5 cm. from the top. Above this point the mediums were clear. Smears showed lanceolate diplococci, a great many round coccus-like bodies, occasionally in short chains, some as large as Staphylococcus albus, others much smaller.

Aug. 28.—Smears from the bottom of the ascites-tissue-fluid culture showed an occasional large diplococcus and chains of diplococci and a large number of very small cocci occurring in clumps, grouped, singly, in twos, and chains of three or four diplococci.

Sept. 15.—The ascites-tissue-fluid culture showed slight cloud throughout the medium. Smears showed a great many extremely small cocci singly, in masses, in twos, and short chains. No large forms were found. Films from a corresponding culture of ascites-dextrose broth showed disintegrating, chiefly gram-negative diplococci and short chains, but no minute forms. Subcultures were made.

Sept. 16.—Blood-agar plates from ascites-tissue fluid showed no growth, and from the ascites-dextrose broth a few green colonies. The subcultures from ascites-tissue fluid into ascites-tissue fluid showed no cloud, while those in ascites-dextrose broth showed a diffuse cloud in the lower one-third of the tube.

Sept. 25.—Smears of these cultures were made again, 10 days after inoculation. The films from ascites-dextrose broth cultures showed ordinary-sized diplococci and streptococci varying somewhat in size (Fig. 12a). The films from the ascites-tissue fluid showed enormous numbers of exceedingly small cocci, diplococci, and short chains together with a few large coccus and diplococcus forms, degeneration forms as described by Flexner and Noguchi (Fig. 12b). Blood-agar plate from culture in ascites-tissue fluid 26 days after inoculation showed a few very small green colonies and a few larger more opaque, grayish colonies. Films of the former showed diplococci resembling pneumococci, of the latter, large coccus and diplococcus forms. Subsequent subcultures from the ascites-tissue fluid on blood-agar were always negative but yielded growths in ascites-dextrose-tissue broth and ascites-tissue fluid, characteristic for these mediums. Exactly similar results were obtained with this strain from the single colony in ascites-dextrose agar after it was passed through 2 guinea-pigs, plated once and then inoculated into ascites-dextrose broth and ascites fluid, plus tissue and oil (Figs. 12c and d).

All shake cultures in dextrose-agar remained free from colonies in this case save the one used for study, and the cultures in liquid mediums showed streptococcus in pure culture. This colony was stabbed Aug. 21 directly into the agar culture and platings showed only typical streptococcus colonies. Sept. 22 this stab showed in smears streptococci and diphtheroid bacilli with all gradations between the two forms in the same chain. The diphtheroid forms were present all along the line of inoculation, but were most numerous in the deeper portions of the stab. Smears at this time from the tube of ascites-tissue fluid inoculated from the single colony showed extremely small streptococci and minute diphtheroid bacilli with all gradations between. Platings at this time on blood-agar showed two types of colonies, one the usual fine, dry colony with green halo, the other a small brownish-gray, indifferent colony. The former showed streptococci, the latter diphtheroid bacilli. Subcultures into broth of the former yielded streptococci, of the latter diphtheroid bacilli. Owing to the close resemblance of the cultures in ascites-tissue fluid to the "globoid" organism cultivated in this medium by Flexner and Noguchi and the filtrability of the virus of poliomyelitis, filtration experiments were done and Sept. 11, 1916, pure cultures of the streptococcus were found in ascites-dextrose broth of a filtrate of a culture in ascites tissue fluid containing small forms from Berkefeld N filter, while the cultures from a dense porcelain filter were negative. Cultures from the cord, after being preserved in 50% glycerin for 3 and 15 months, yielded pure cultures of the streptococcus.

Sections of the cord showed typical poliomyelitic findings, moderate round-cell infiltration of the pia, particularly over the anterior aspect of the cord and in the anterior fissure, marked dilatation of vessels, marked round-cell infiltration and degeneration of ganglion cells in anterior horns and moderate perivascular infiltration (Fig. 1). Search for bacteria in sections revealed gram-staining cocci and diplococci of various sizes in the pia and in the anterior horns of cervical cord and medulla (Fig. 2).

Case 712.—L. K., a girl, aged 4 years, was admitted to the New York Hospital Aug. 22, 1916. No accurate history was obtainable but it was learned that illness began 4 days before admission. On admission the patient was drowsy, scarcely able to speak or breathe, and was unable to lift her head. There was weakness

of the right shoulder and neck, flaccid paralysis of both lower extremities with the exception of slight flexion of the toes of the left foot, and absence of abdominal and knee reflexes. The spinal fluid was found to be under moderate pressure, slightly turbid, and 40 c c were withdrawn; there were 350 cells per cubic millimeter. Paralysis of the respiratory muscles developed and progressed rapidly, and death occurred Aug. 23.

At necropsy there was found edema surrounding the dura, especially in the cervical region, extending along the nerve roots and ganglia. The ganglia appeared edematous. The spinal fluid was clear and increased in amount. There was marked hyperemia of the vessels in the pia. There were hemorrhages and edema in the gray matter of the cord, especially in the anterior horns. The brain could not be obtained. The peritoneal cavity contained a small amount of clear fluid. The stomach contained a moderate amount of chocolate-colored fluid free from food. The mucous membrane was studded with numerous bleeding points, especially in the fundus, some of which showed superficial ulceration. The spleen was firm; the cut surface was moist. The lymph follicles were prominent as round, opalescent, grayish points. Kidneys, liver, adrenals, pancreas, pelvic organs, intestines, appendix and thymus showed no noteworthy changes. The tonsils were of normal size. Numerous cross-sections made with a razor, beginning at the base, showed a small abscess in each tonsil containing a peculiar semiliquid, opalescent pus. Cultures were made from the liver, spleen, kidney, spinal cord, and pus from an abscess in the tonsil. Those of the liver, spleen and kidney were sterile. Cultures of emulsions of the cord showed gram-positive cocci of varying size singly, in twos, and short chains. Blood-agar plates from cultures of cord showed two types of colonies, rather large grayish-white colonies resembling Streptococcus albus, but less opaque, and numerous smaller colonies resembling Streptococcus viridans. Ascites-dextrose-broth culture of tonsil pus showed short-chained streptococcus and large cocci singly, in pairs, clumps and short chains. Blood-agar plate of tonsil pus showed chiefly grayish-white colonies resembling staphylococcus colonies and nonhemolyzing colonies of streptococci.

Sections of the cord showed extreme perivascular and diffuse infiltration, chiefly of gray matter of the cord, medulla and pons. The perivascular infiltration was especially prominent around the vessels extending in from the surface of the cord. There was moderate infiltration of the pia, especially anteriorly, and in the anterior fissure, and marked degeneration of ganglion cells (Fig. 3). Sections of the viscera showed no changes other than hemorrhages in the mucous membrane of the stomach, and small areas of necrosis with aggregations of round cells in the liver. No bacteria could be detected. Fully 40 gram-staining diplococci or cocci of various sizes were found in the anterior horns of the cord, chiefly at the peripheral zone of circumscribed areas of cellular infiltration, in perivascular areas of infiltration in the pia and in the edematous dura, and in infiltrated ganglia and hemorrhagic areas surrounding the ganglia.

Sections of the tonsils showed a number of areas of necrosis in the center of the lymphoid follicle near the base of the tonsil, and a small amount of exudate in the crypts. The cells in the necrotic areas were made up chiefly of large and small mononuclear cells, but polymorphonuclear leukocytes were also present. The cryptic exudate contained many leukocytes and large mononuclear cells and debris. In the areas of necrosis numerous gram-staining cocci were found which varied greatly in size. Some were very large, singly or in twos, while others were exceedingly small, singly, in twos and in short chains. Large forms were found which showed all grades of division. Some were perfectly round, others only slightly elongated, some were breaking into 2 and each of these again divid-

ing into smaller diplococci. Other round forms appeared to be breaking into 4 cocci, each of which in turn showed beginning division. Others were breaking into 8 small cocci, arranged in pairs, while still others were dividing into 8 diplococci and each of these showed a light line of beginning division too small to be photographed or drawn. Chain formation was slight but evident in some fields and appeared to be the result of transverse division of larger coccus forms in some instances. Both Gram and eosin, and polychrome methylene blue stains gave this picture (Fig. 10). Bacilli were not found in these areas. The exudate in the crypts was composed of necrotic cells and showed numerous gram-staining diplococci and short chains, large coccus forms and a moderate number of fusiform bacilli. Relatively few bacteria were found in parts of the tonsils showing no lesions, although cocci and diplococci of varying size, but always smaller and more streptococcal in shape and size, were found in perivascular areas of infiltration even beyond the capsule, along sheaths of muscle fibers, within capillaries, and in blood vessel walls.

Case 714.—J. B., a girl, 2 years of age, was admitted to the New York Hospital, Aug. 13, 1916. Four days before admission she had fever, diarrhea, drowsiness and loss of appetite. On the 3rd day marked weakness of both legs appeared. On admission there was complete flaccid paralysis of both legs and left arm, with absence of abdominal and knee reflexes.

Aug. 22, the respiration became difficult and irregular. The following day respirations were wholly diaphragmatic, the temperature became higher, and edema of the lung and bronchopneumonia became evident. Death occurred Aug. 24.

The spinal fluid on admission was clear; 20 c c were withdrawn and 30 cells per cubic millimeter were found. Aug. 22, 14 c c of clear fluid were withdrawn and 4 cells were found.

At necropsy edema was found surrounding the dura, nerve roots and ganglia, especially along the cervical and lumbar enlargements. The ganglia appeared swollen and edematous. The cerebrospinal fluid was clear but increased in amount. Cross-sections of the cord showed marked edema and softening and small hemorrhages in areas in the anterior horns. The pial vessels over brain and cord showed extreme congestion. The pia arachnoid was edematous and showed a number of hemorrhages over the anterior aspect of the medulla. The peritoneal and pericardial cavities contained a moderate amount of clear fluid. There was moderate cloudy swelling of liver, kidney, and myocardium. The spleen was firm, the lymph follicles were prominent and the cut surface moist and bloody. The right lung was firm, mottled grayish-red and free from crepitation, with the exception of the anterior portion of the upper and middle lobes. The other viscera were normal. The tonsils were slightly enlarged but the surface appeared normal. Numerous cross-sections, beginning at the base, showed not less than 6 abscesses situated near the base, varying in size from 1-3 mm. All contained thick, opalescent, gray-yellow pus. The crypts contained a few small, firm plugs. Cultures of the tonsils and pus from the abscesses in the tonsil showed enormous numbers of fine, dry colonies of streptococci surrounded by a green zone and a few larger, more opaque colonies resembling Staphylococcus albus. No hemolytid streptococci were found. Cultures from the lung showed pure growth of diplococcus resembling pneumococcus. Those from the cerebral fluid, the edematous fluid surrounding the dura of the cervical cord, the cervical cord and the brain showed pure growths of green-producing, short-chained streptococcus varying greatly in size and shape. Cultures from the kidney were sterile. Those from the blood showed diphtheroid bacilli.

Numerous parallel cultures were made with the streptococcus isolated from this case in ascites-dextrose broth with and without tissue, and in ascites-tissue fluid plus oil. The character of growth in these mediums was studied also after the strains were plated and single colonies used for inoculation after they were passed through from 1-4 animals and after the brain of the animals and cultures containing the small forms were filtered. In all instances, if growth occurred, it was characteristic of these mediums, independently of the age of the culture (Fig. 13a). In the former it resembled streptococci or pneumococci with some irregularity in size and grouping. In young cultures there was usually a slight unstained zone surrounding the diplococci suggesting a capsule, while in the ascites tissue fluid the organism always became small in size and tended to lose the stain in Gram's method (Fig. 13b).

Sections of brain and cord showed typical changes of poliomyelitis, dilatation of vessels and hemorrhages in anterior horns, marked degeneration and slight phagocytosis of ganglion cells, moderate perivascular and diffuse infiltration in the gray matter of the cord, slight in brain pia and spinal ganglia. Sections of the tonsils showed hyperplasia of lymph follicles but no areas of necrosis. A moderate number of diplococci and cocci of various sizes were found in the lymph follicles and many in the crypts together with fusiform bacilli. Sections of viscera showed no noteworthy changes with the exception of the lung where hemorrhage and leukocytic infiltration were marked. Search for bacteria showed large and small cocci and diplococci in smears from the anterior horn and surface of cerebrum, and in sections of cord and intervertebral ganglia (Fig. 5). No bacteria were found in sections of kidney, spleen, myocardium and pancreas.

THE TONSILS AND ADENOIDS

The affinity of the virus of poliomyelitis for lymphoid tissue has been emphasized and so-called virus has been demonstrated in the tonsils. Notwithstanding these facts, however, little attention has been given to a study of the tonsils and adenoid tissue to determine the presence or absence of lesions, the number and kind of bacteria in the tissues, and the importance of these structures as affording entrance for the infective agent in poliomyelitis. At the very outset of this study, one of us was impressed by the fact that a relatively large amount of infective material could be expressed from tonsils which, on inspection, appeared quite normal. Peabody, Draper and Dochez found the tonsils and lymph glands enlarged in a high percentage of cases in their series, but appear not to have studied the tonsils after death. Microscopic examination of the material expressed and cultures made by us revealed the presence of cocci and streptococci which showed marked variations in size and shape (Fig. 11). Deep cultures in ascites-dextrose broth of the very first case studied (July 21, 1916) showed[21] "apparently pure cultures of streptococci in short and medium chains and in pairs. Some of the diplococci were large and lanceolate in shape, resembling pneumococci, and others were

small and more coccus-like. Both large and small forms were found
in the same chain."

Young rabbits and guinea-pigs (animals considered resistant to
poliomyelitic virus) injected intravenously with freshly isolated cul-
tures, developed flaccid paralysis.[20] These findings were strikingly
different than had been obtained in a study of tonsils in other diseases
and suggested the possibility that the "globoid" organism described by
Flexner and Noguchi as the cause of poliomyelitis might be a form
of streptococcus, and hence a detailed study of this question was
undertaken.

Blood-agar plate cultures of the pus expressed from tonsils at the
beginning of the attack as illustrated in the protocols, showed almost
constantly large numbers of fine, dry, green-producing colonies of
streptococci and a small but variable number of larger, more opaque,
glistening colonies resembling staphylococci; usually a few small,
slightly hemolyzing colonies of streptococci, a moderate number of
Micrococcus catarrhalis and occasionally a few colonies of colon bacilli
and other extraneous organisms. Typical hemolytic streptococci were
almost never found except in cases which showed a complicating fol-
licular tonsillitis. Cultures from abscesses of tonsils removed before
and after death showed with remarkable constancy this same type of
flora and frequently showed only the two first types of colonies. Cul-
tures from single fine, dry, green colonies in ascites-dextrose broth
often showed remarkable variations in size and shape (Fig. 11), and
if again plated, frequently showed the fine, dry, green and larger,
more opaque colonies, especially from old cultures.

In 17 cases, the patients ranging in age from 7 months to 24 years,
in which the tonsils were examined after death by making numerous
cross-sections with a razor, from 1-15 abscesses were found. These
were usually situated near the capsule and were not connected with
the crypts. They varied in size from 1 or 2 mm. to 5 mm. in diameter
and usually contained a thick opalescent grayish-yellow material,
softer and more opaque than the collections of cells from swollen fol-
licles. Smears showed large and small mononuclear cells, leukocytes,
debris, and later enormous numbers of diplococci of medium size,
large and small coccus and diplococcus forms, and occasionally fusi-
form bacilli. The small forms resembled "globoids," but no sharp line
could be drawn between these and the larger forms; all types were
often seen in the same chain. On the basis of these striking findings,

and after it had become evident that the organisms were of low virulence, it was determined through the cooperation of Dr. J. C. Roper to remove the tonsils in a series of patients who were not convalescing satisfactorily and in whom a low grade of fever persisted. This was done in 11 cases. In 3 patients there was a slight temporary rise in temperature, in 5 the temperature curve was not altered and in 3 it was definitely lowered. Seven of the patients showed improvement in their general condition, they were brighter and less irritable. In the others no noticeable change occurred.

During tonsillectomy a large amount of infected material was noted, and in all, one or more small abscesses were found in the tonsils on making sections. These abscesses were less prominent than in the fatal cases. Similar abscesses and identical organisms were found in adenoids removed before and after death. Sections of the tonsils showed marked hyperplasia of the lymph follicles and in all but 2 of the 14 cases studied, one or more small or large areas of necrosis in lymph follicles (Table 1 and Fig. 8). The exudate in the crypts contained leukocytes, round cells and many cocci and diplococci and moderate numbers of fusiform and other bacilli. In a number of instances the epithelial lining of the crypts showed atrophy but usually this could not be made out.

The cells in the necrotic areas in the follicles were chiefly large and small mononuclears, although in some instances a large number of leukocytes were present. Many large and small cocci and diplococci exactly like those found in small numbers in the brain and cord and mesenteric glands were found in these areas. Bacilli were almost wholly absent. Excellent opportunity to study the relationship between large and small forms was here afforded and in a number of instances unmistakable evidence of large coccus and diplococcus forms breaking into the smaller cocci were discovered (Figs. 9 and 10). The number of bacteria demonstrable within swollen follicles was surprisingly small. Those found corresponded very closely in size and shape to the cocci found in the cord and mesenteric lymph glands. Bacilli were never found in swollen but otherwise normal follicles. In a number of instances a large number of cocci and diplococci were found in areas of round-cell infiltration, in the capsule of the tonsil, and around large blood vessels outside the capsule. A few were found in blood vessel walls and within the lumen of blood vessels.

In the Davenport epidemic a study of the relation of infection in tonsils to enlargement of cervical lymph glands immediately outside

the tonsils was made in 38 cases. In 26 of these the cervical glands were enlarged, and in 11 the enlargement on either side was in proportion to the amount of infection in the tonsil on the corresponding side, as determined by the greater size of the tonsil or larger amount of infected material which could be expressed. In several instances these findings were verified at necropsy. No definite relation between infected conditions of the teeth could be made out although caries was present in some of the cases. The tonsils from 12 persons removed for various reasons in other diseases have been examined in the same way. The abscesses in the lymph follicles on cross section and the areas of necrosis noted microscopically in poliomyelitis were not found, although abscesses in crypts containing many organisms with atrophy of the epithelium were found in some of these.

The results of the study of the tonsils and adenoids here reported indicate strongly that these structures afford important entrance ways for the micro-organisms which we find constantly in the diseased tissues in poliomyelitis. A relatively large amount of infected material may be expressed from the tonsils. The abscesses and areas of necrosis are more numerous and are larger in the fatal cases than in tonsils removed from convalescent patients. These abscesses contain large numbers of this micro-organism, particularly in the fatal cases, and the cocci have been demonstrated in tissues immediately outside the tonsils, in areas of infiltration along large blood vessels and within the lumen of capillaries. Injection of cultures into animals produced flaccid paralysis. Removal of tonsils and adenoids in convalescent patients has been followed by seemingly good effects in some instances. In view of these facts there can be little doubt but that diseased tonsils and adenoids predispose to poliomyelitis and may lead in some instances to severe or fatal infection which might otherwise be mild. The focal areas of infection with abscess formation in these structures may be an important factor in prolonging an attack, in preventing early restoration of lost muscle function and be the cause of remissions or relapses which occur not infrequently as pointed out especially by Wickman and by Draper. The abscesses are usually small, and so situated mechanically as not to interfere with their healing. Protection by removal of these structures is only relative at best and temporary entrance channels for bacteria are made by their removal. Hence, their wholesale extirpation for protection against this dread disease would not be justified. Their removal in special instances may be

useful, but the well established principles for the removal of tonsils and adenoids as applied in connection with other conditions should be followed in this disease.

SUMMARY

A pleomorphic coccus has been isolated from and demonstrated in affected tissues in all the cases examined of poliomyelitis that occurred in different epidemics and in widely separated parts of the country. The organism has been found in large numbers in adenoids and tonsils, in smaller numbers in lesions in various parts of the central nervous system, and in the mesenteric lymph glands. It has been proved to be absent in organs showing no lesions. The organism shows great variations in size and shape, depending on the method of cultivation. In aerobic cultures, although more irregular in size, it closely resembles ordinary green-producing streptococci or pneumococci of low virulence. Under anaerobic cultivation, especially in tall columns of ascites fluid plus tissue and oil, it grows to very small size, becomes filtrable and in every way appears identical with the "globoid" organism described by Flexner and Noguchi. The variations noted in cultures have been proved not to be due to contaminations or to mixed cultures. All gradations in size between large coccus or diplococcus forms to exceedingly small, almost ultramicroscopic forms, were found alike in cultures and in the tissues, and the conditions favorable for the formation of one from the other have been determined. Morphologic evidence of the breaking down of large forms into the small forms in the tissues has been obtained. Pure cultures of this organism have been isolated many times and it has been demonstrated in films and sections of brain and cord many months after they were placed in 50% glycerol. In view of these results the presence of this organism in the diseased tissues cannot be considered an accidental contamination.

The following facts, determined since the studies reported in this paper were begun, indicate that the organism here described bears etiologic relationship to poliomyelitis. It is constantly present in the diseased tissues, from which it can be cultivated even many months after glycerolation. On injections of cultures into young rabbits and guinea-pigs it localizes specifically in the nervous system and produces flaccid paralysis and changes in brain and cord which resemble those in poliomyelitis in man.[20] From the brain and cord of these animals the organism can be isolated and the disease again produced. The organism has been rendered filtrable. By means of the same methods

the identical organism has been isolated constantly from the brain and cord of monkeys paralyzed with fresh, glycerolated and filtered virus.[19] The serums of persons and of monkeys, having recovered from poliomyelitis, agglutinate specifically the more sensitive strains both from human and monkey poliomyelitis.[18] Injections of the recently isolated aerobic cultures into monkeys renders them refractory to virus. The aerobic form of the organism from human and monkey poliomyelitis produces antibodies in the serum of horses, in a large amount common for both, cross-agglutinating these strains specifically in high dilution.[19] The serum of a horse immunized with freshly isolated strains from monkeys protected monkeys relatively against intracerebral inoculation of virus[16] and had pronounced curative effects in the treatment of human poliomyelitis. Early intravenous injections were followed by almost immediate cessation of symptoms in a large series of cases.[17]

The results of Flexner and Noguchi, so far as the cultivation of a small filtrable organism and its demonstration in the tissues in poliomyelitis are concerned, have been corroborated, but the results of our experiments indicate that this is the anaerobic and, according to Amoss' results, a nonantigenic form of the organism which, under aerobic cultivation, clearly belongs to the streptococcus group of microorganisms. Both forms have been constantly demonstrated side by side in the tissues of poliomyelitis. Flaccid paralysis coming on soon after injection has been produced in monkeys with characteristic, although not typical changes in the cord with aerobic cultures, but the classic picture as obtained with virus in this species has not been secured . It may be suggested, however, on the basis of results already obtained, that this is due to the development of antibodies, since the organism in the aerobic form has marked antigenic powers.

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EXPLANATION OF PLATES 6-8

Fig. 1.—Section of upper segment of cervical cord (Case 707) showing typical poliomyelitic lesions. For explanation of letters see Fig. 2. Hematoxylin and eosin. × 20.

Fig. 2.—Micro-organisms in section of cervical cord shown in Fig. 1. Gram-Weigert. × 1000. Large and small diplococcus in edematous pia at *a*. Medium-sized diplococcus in pia at *b*. Small diplococci and cocci in anterior horn adjacent to blood vessel at *c*.

Fig. 3.—Section of cervical cord (Case 712) showing typical poliomyelitic lesions. For explanation of letters see Fig. 4. Hematoxylin and eosin. × 50.

Fig. 4.—Micro-organisms in cervical cord (Case 712) shown in Fig. 3. Gram-Weigert. × 1000. a. Large diplococcus adjacent to blood vessel at *a*. b. Medium small diplococcus, round cells and leukocytes at periphery of area of infiltration at *b*. c. Medium-sized diplococci in pia. d. Medium-sized diplococcus in ganglion at *d*.

Fig. 5.—Micro-organisms in nervous system of Case 714. × 1000. (a) Large and small elongated cocci in film from anterior horn. Giemsa. (b) One large coccus, medium-sized diplococci and very small diplococci in contact film near surface of cerebrum. Giemsa. (c) Large and small diplococci in edematous infiltrated area in anterior horn of cervical cord. Gram-Weigert. (d) Diplococcus in intervertebral ganglion. Gram-Weigert.

Fig. 6.—Micro-organisms in nervous system, Case 729. × 1000. (a) Diplococcus in contact film of cerebrum. Giemsa. (b) Large coccus form breaking into small diplococci and cocci, and a small lightly stained diplococcus in contact film of cerebrum. Giemsa. (c) Two medium-sized diplococci in chain and small diplococcus in pia near anterior fissure. Gram-Weigert. (d) Three diplococci in pia anterior fissure. Gram-Weigert. (e) Diplococcus adjacent to leukocyte in edematous area in anterior horn of lumbar cord. Gram-Weigert.

Fig. 7.—Micro-organisms in nervous system in cases of poliomyelitis. × 1000. (a) Group of small, lightly stained diplococci in infiltrated area surrounding blood vessel, Case 779. Gram-Weigert. (b) Diplococci in normal salt solution emulsion of cervical cord, Case 745. Gram-Weigert. (c) Chain of three diplococci in smear from beneath dura of cord of Case 949 after preservation in glycerol for 4 months. Giemsa.

Fig. 8.—Necrosis of lymph-follicle near base of tonsil, Case 712. Hematoxylin and eosin. × 40.

Fig. 9.—Large coccus and medium-sized diplococcus at *a* (Fig. 8), the former breaking into four small diplococci. Gram-Weigert. × 1000.

Fig. 10.—Composite drawing of various levels of field shown in Fig. 9, to show details of relation between large and small forms of micro-organisms and the mechanism of formation of the small forms.

Fig. 11.—Smears from 18-hour cultures in tall tubes of ascites-dextrose broth inoculated with single colonies of green-producing streptococcus from blood-agar plate inoculated with pus from tonsil, Case 695. Note the extreme variations in size, shape and grouping, and various stages of division of cocci in chain in *c*. Lanceolate diplococci undergoing fission into flat-sided diplococci and further longitudinal fission resulting in small forms. Gram. × 1000.

Fig. 12.—Smears from aerobic and anaerobic cultures of streptococcus isolated from the cord of Case 707. Gram-Weigert. × 1000. (a) Twelve-day culture in ascites-dextrose broth in third culture and derived from single colony in culture from cord, showing streptococcal type of growth. (b) Same as *a* but from tall tube of ascites-fluid plus tissue and oil, showing very small cocci, diplococci and short chains. (c) Same strain after two animal passages and from single colony on blood-agar plate. Forty-eight hour culture in tall tube of ascites-dextrose broth showing typical streptococcal type of growth. (d) Same as *c* but in ascites fluid plus tissue and oil after incubation for 24 days, showing extremely small cocci, diplococci and short chains and one large "degeneration" form.

Fig. 13.—Smears from cultures of streptococcus isolated from the brain in Case 714. Gram. × 1000. (a) Six-day culture in ascites-dextrose-tissue broth from brain of monkey paralyzed with culture in the fourth animal passage. Note quite typical streptococcal forms. (b) Same as *a* but culture in tall tube of ascites-fluid plus tissue and oil. Note small micrococcus and diplococcus type of growth. (c and d.) Eighteen-hour culture in ascites-dextrose broth of above strain after one plating and after five successive anaerobic cultures in ascites-tissue fluid. Note the extreme variations in size and shape and the yeast-like bodies occurring singly and in chains of typical streptococci.

PLATE 6

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PLATE 7

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PLATE 8

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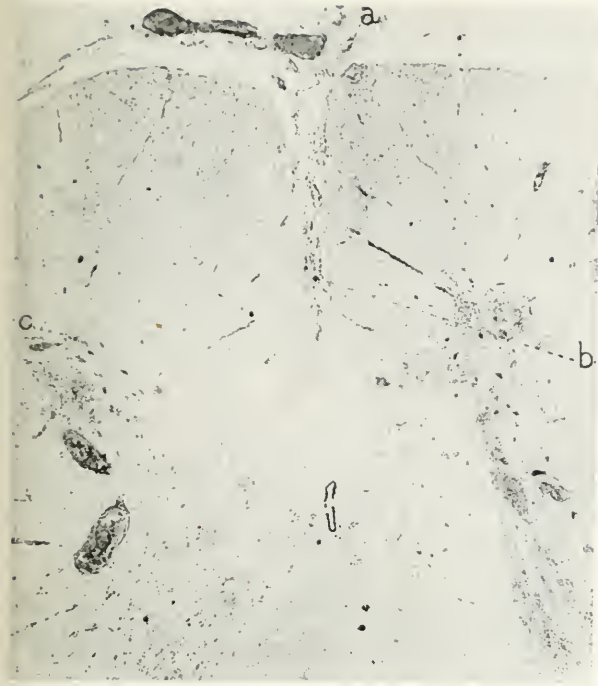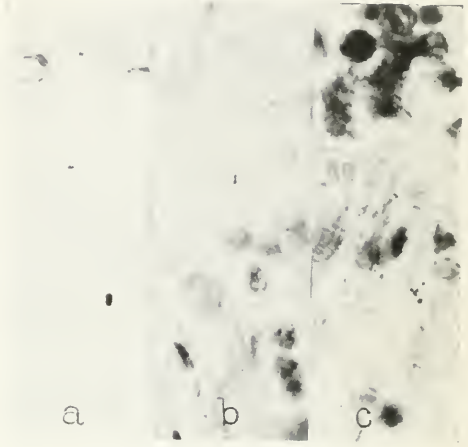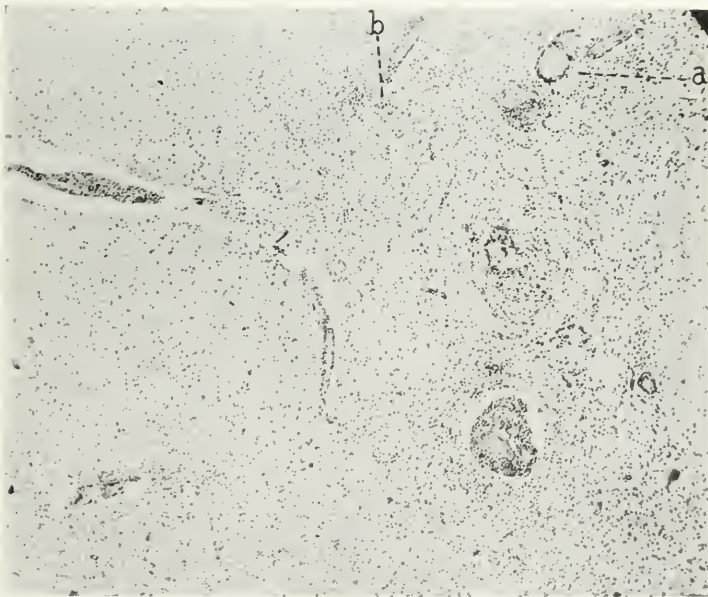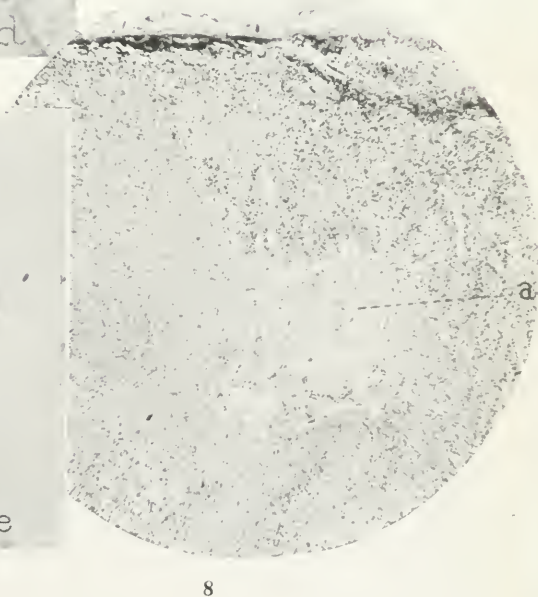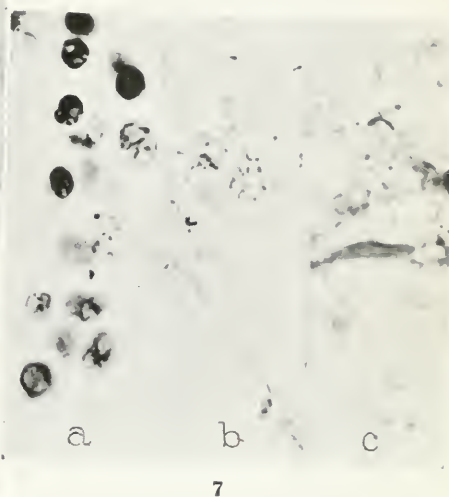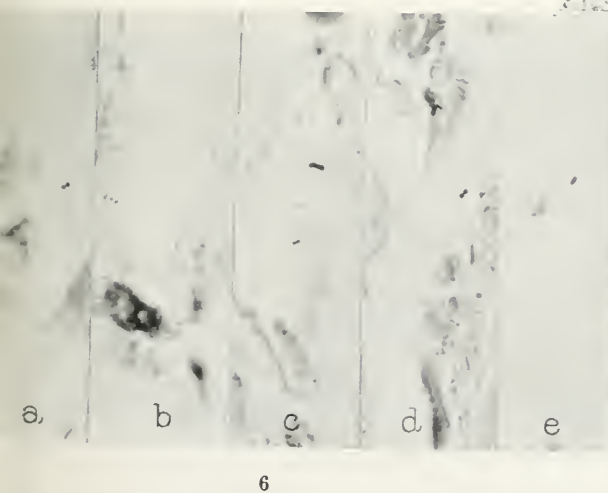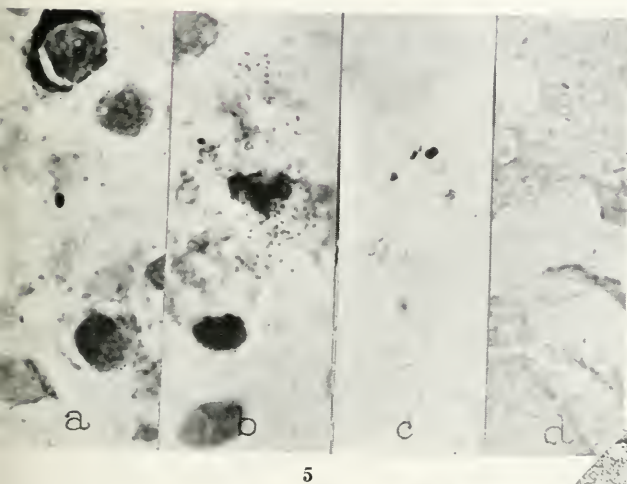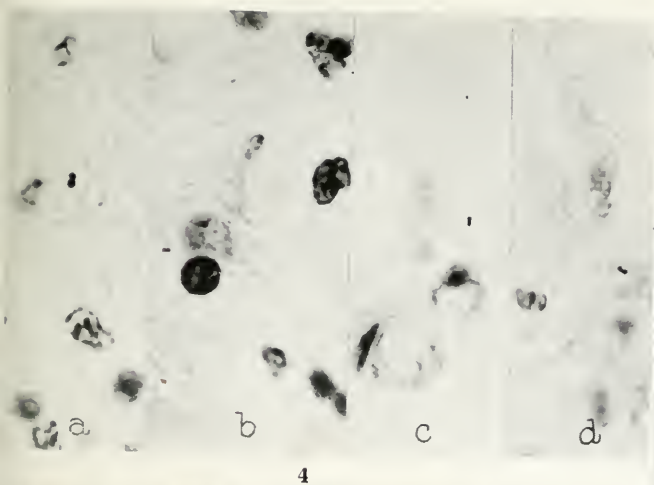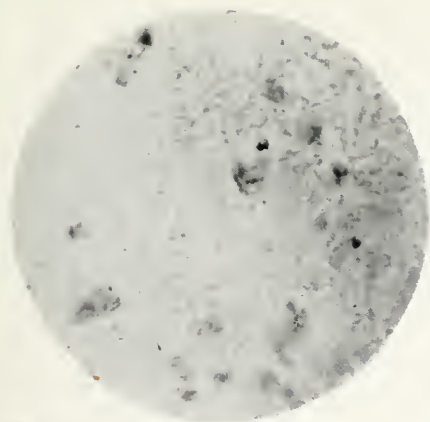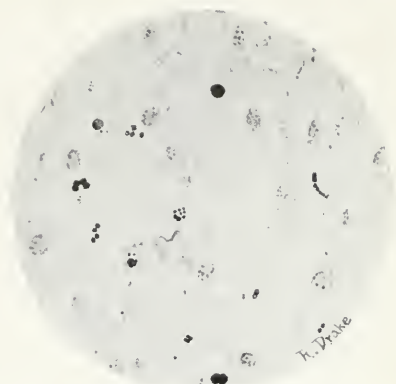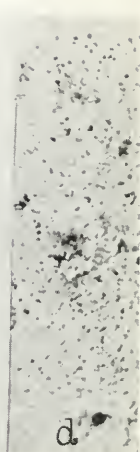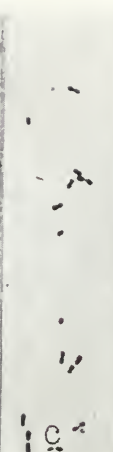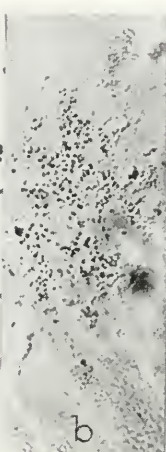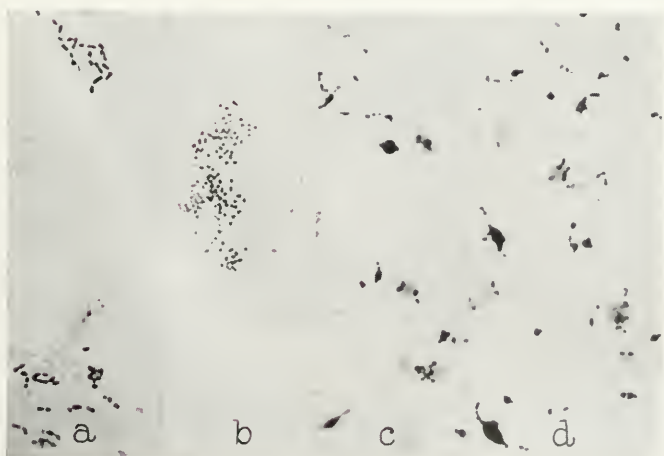
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THE ELECTIVE LOCALIZATION OF STREPTOCOCCI FROM EPIDEMIC POLIOMYELITIS

PLATES 9-13

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It is our purpose in this paper to review the bacteriology of poliomyelitis, to record experiments on the elective localizing power of bacteria isolated from the supposed infection-atrium and infected tissues in cases of poliomyelitis which occurred during the epidemic of 1916, and to discuss briefly the significance of the findings.

Many attempts have been made to produce lesions in the central nervous system, especially myelitis, by the intravenous injection of bacteria. Roger injected streptococci in rabbits. Emaciation and muscular atrophy resulted, and there was degeneration of the ganglion cells in the spinal cord, but bacteria were not demonstrated. Bourges, working with the streptococcus from erysipelas, discovered that one of his rabbits developed paraplegia, paralysis of the sphincters, and muscular atrophy. At necropsy, 15 days after inoculation, no lesions were found except in the spinal cord which showed hyperemia, hemorrhages, neurogliosis and nerve-cell degeneration, but no streptococci. Morel and Rispal had one case of experimental myelitis in which, 7 days after inoculation, streptococci were found both in the blood and in the spinal cord. In a large series of animals (116), Vidal and Besanzon observed paralytic symptoms in seven. Only degenerative lesions of the spinal cord were found. No streptococci were discovered in sections or cultures. Poynton and Paine recovered streptococci from the cerebral fluid in one rabbit after intravenous injection. No pathology or symptoms were mentioned. Wickman[51] attempted to produce a special affinity of streptococci for the central nervous system in rabbits by repeated passage through the central nervous system, but failed. Similar results were obtained by Marinesco. Hoche and others could obtain lesions of the spinal cord on intravenous injection of bacteria only by the simultaneous injection of inert powders.

Davis, Horder, Henrici, Moody, Irons, Brown and Nadler, Gerdine and Helmholz, Hardt, Detweiler and Robinson, Blake and others, who have injected numerous animals intravenously with streptococci from sources other than the nervous system, have not reported the occurrence of flaccid paralysis due to lesions in the central nervous system. Bull produced meningitis in dogs by injecting intravenously large doses of virulent pneumococci. In other animals paralysis, apparently of the spastic type, developed, but the occurrence of flaccid paralysis is not mentioned.

During elective localization studies made by one of us,[44, 45, 46] some 2,000 rabbits and guinea-pigs were used, excluding those injected with bacteria isolated from diseases of the nervous system. Sixty per cent

of these animals lived from 1-3 days, 20% from 3-7 days, 10% from 7-20 days, and 10% 20 days or longer. Only three showed definite paralysis, which was proved not to be a general weakness due to over-whelming infection.

Weakness, especially of the hind extremities, shortly before death was noted occasionally, but this was taken to be a part of an over-whelming infection.

It is evident, therefore, that bacteria obtained from miscellaneous sources rarely localized in the central nervous system following intra-venous injection. Even in some of the experiments considered positive, paralysis alone was the basis of diagnosis with no necropsy to rule out other causes. Frequently the microscopic examination was unsat-isfactory and bacteria usually could not be found. The concensus of opinion has been ably expressed by Buzzard: "The whole of these researches illustrate the difficulty which is encountered in producing experimentally an infective condition of the central nervous system through the general circulation, an experience which is not without parallel in clinical medicine, when we consider how comparatively rarely ordinary pyogenic organisms are the cause of disease within the brain and particularly within the cord unless as a direct infection from the neighboring tissue."

The isolation of bacteria in acute poliomyelitis has been reviewed by various authorities (Wickman,[51] Landsteiner, Römer[41] and others). Schultze, in 1898, found diplococci in the spinal fluid by spinal puncture in a case 13 days after the onset. Cultivation on glycerin agar was negative. Following this, Bülow-Hanson and Harbitz, Concetti, Looft and Dethloff, and Engel, each of whom reported one or two cases, found a similar organism.

Giersvold, who studied the epidemic in Norway in 1905-1906, isolated a gram-positive polymorphic diplococcus from the spinal fluid in each of 12 cases of poliomyelitis, in some instances both before and after death. He noted that the later in the disease the spinal puncture was performed the fewer were the bacteria. After from 12-14 days the spinal fluid was often sterile. He stated further, that the isolated microorganisms, except in one case in which there were also pneumo-cocci, were obtained in pure culture, that they were bean-shaped diplococci or tetracocci, often in clumps when grown on solid mediums, and in chains of 4-6 diplococci when grown in liquid mediums, the line of division between the pairs corresponding with the axis of the chain, and that the growth in the first culture generation was usually slight but became richer on transplantation. Paralytic symptoms were noted following injection in mice and rabbits in a few instances.

Fox,[12] in 1907, found gram-positive and biscuit-shaped diplococci and tetracocci in the cultures from the nose and throat of all his acute poliomyelitis cases. The spinal fluid which was obtained in four instances showed the same organism. In another epidemic in 1910,[13] the gram-positive diplococcus was found in smears from the inner portions of the brain and cord of 3 cases in man, where, it is

said, "there could be no possibility of contamination from the outside." Pasteur, Foulerton and MacCormac found the same organism in the spinal fluid in one case. No intravenous inoculation into animals was made.

Potpeschnigg, in 14 cases, found the same diplococcus both in smears and cultures of the spinal fluid although later he decided they were contaminations. In 1910, "Giersvold's diplococcus" was found by Robertson and Chesley in the spinal fluid in 7 cases and also in the brain in one fatal case. Three-tenths of a c c of a growth from agar-slants injected intravenously into each of several animals, gave paralytic symptoms in two. Poliomyelitic lesions were not found in the spinal cords. The same diplococcus was recovered from the cerebrospinal fluid of some of their animals.

About this time Landsteiner and Popper, Flexner and Lewis, Römer,[12] and Leiner and Wiesner, each succeeded in producing typical poliomyelitis in monkeys with virus, and later with filtrates of virus. Flexner and Noguchi, in 1913, cultivated minute "globoid" bodies from the brain and cord which, when injected intracerebrally in monkeys, produced typical lesions of poliomyelitis, and which they state "fulfill the conditions hitherto demanded for the establishment of causal relationship between an extraneous parasite and a specific disease." They considered cultures contaminated when containing bacteria of ordinary size. To quote: "Any tube showing marked turbidity, coagulation, or gas-production within one or two days may be set aside as grossly contaminated. The tubes which remain clear or show only slight turbidity may none the less be contaminated. To exclude contamination, small quantities of the medium are removed with pipettes and stained for bacteria in the ordinary way and cultivated on the usual solid or fluid media. Obvious bacteria in the films as well as any growth taking place in the ordinary media in the presence of air, may be put down as contaminations and the tubes containing them discarded."

From that time, bacteria of ordinary size have been considered of little or no importance in the etiology of acute poliomyelitis.

In attempting to determine causal relationship of the bacteria isolated from poliomyelitis, investigators have usually injected relatively small doses of culture from agar slants or from low columns of bouillon. Little or no attention was paid to the question of oxygen-tension, temperature of incubation, and the length of time organisms were grown on mediums before injection. The importance of these factors has been emphasized in articles by Rosenow[44, 45, 46] in connection with a study on the etiology of various diseases, including diseases of the nervous system. By affording bacteria a gradient of oxygen-pressure at a relatively low temperature (33-35 C.), and by injecting a large number of organisms soon after isolation, there was observed

a marked tendency for the bacteria to localize and produce lesions in the tissues of animals corresponding to those involved in the spontaneous disease.

On the basis of these experiments the completely negative or largely negative results of the earlier investigations were considered inconclusive as proving that the diplococci or streptococci found by many observers in poliomyelitis, were contaminations and therefore of no etiologic importance. Accordingly it was decided to reinvestigate the bacteriology of epidemic poliomyelitis and to determine any specific localizing power of the bacteria isolated by means of the newer methods.

From July 21 to Aug. 12, 1916, the localizing power of cultures from tonsils in 7 cases of poliomyelitis that occurred in Rochester, Minn., were studied. Paralysis and corresponding spinal lesions were produced in some animals with cultures from all 7 cases (Figs. 1, 2, 10 and 14). A pleomorphic streptococcus was found to be the organism which showed specific localizing power. On the basis of these results the work was continued in New York where postmortem material might be studied.* The experiments with tonsil-strains were repeated with similar results and Aug. 21 the first series of animals was injected with the pleomorphic streptococcus isolated in pure culture from the spinal cord in a typical case of poliomyelitis (Pig 324). A description of the pleomorphic streptococcus isolated from the infection-atrium in numerous cases and from the central nervous system or lymph glands in each of 12 cases and its elective affinity for the nervous system was given in a preliminary report.

Mathers,[30] and Nuzum and Herzog have reported similar results using methods which embody essentially the points which had been emphasized repeatedly by Rosenow in elective localization studies, even to the point of incubating cultures for injection at a relatively low temperature (35 C.).† Mathers[31] reported definite paralysis in 10 of 18 young rabbits injected with cultures from 1 case, and gross and microscopic changes similar to those in acute poliomyelitis in man in 15. Hektoen has shown that the microscopic changes are indeed quite like those in man. Nuzum and Herzog inoculated 50 rabbits and reported the development in a considerable number of "a most striking type of progressive flaccid paralysis involving both hind legs, the front legs,

only one leg, or, in 3 cases, all four legs." Histologically, the changes observed by them "differ from the human in that no perivascular infiltration is present and that the types of infiltrating cells are probably not lymphocytes." Flaccid paralysis was also produced in young dogs but repeated injections in guinea-pigs resulted negatively in their hands. That the positive results obtained by Rosenow, Towne and Wheeler,[18] by Mathers, and by Nuzum and Herzog are due to the methods employed is supported by the fact that Bull, and Kolmer, Brown and Freese[22] have recently reported negative results obtained by methods similar to those used by the earlier investigators and which do not take into account sufficiently the points essential for elective localization. Thus, the results obtained by Kolmer, Brown and Freese may be accounted for by the aerobic cultivation of the bacteria, the smallness of the dose and the use of adult rabbits (1,400-1,600 gm.).

<div align="center">TECHNIC</div>

The technic was essentially that used in elective localization studies in other diseases. Painstaking effort was made to express the material containing the bacteria from the depths of the tonsils. This was done by means of a small laryngeal mirror straightened to an angle of about 30 degrees with the holder. The material was scooped out and then swabbed from the mirror with a sterile cotton swab. Surface bacteria were eliminated as far as possible by having the patient drink water or gargle with some antiseptic solution just before examination. Extirpated tonsils were thoroughly washed in sterile salt solution and then cut into thin slices and emulsified in salt solution. The infected material from abscesses was suspended in salt solution and then inoculated into the various mediums, and in some instances directly injected into animals. The infected tissues, brain, spinal cord and lymph glands, were obtained in a sterile condition, emulsified usually in a sterile air chamber and then inoculated into mediums and, in some instances, injected intracerebrally into animals.

The bacteria for injection were usually grown under partial oxygen-tension in tall columns (9-10 cm.) in bottles, approximately 150 c c capacity, of ascites-dextrose, or of dextrose broth (0.2% dextrose; 0.6% acid to phenolphthalein) with or without tissue. The cultures were incubated at from 33-35 C. for from 18-24 hours and occasionally longer. The primary culture was chiefly used, although the second, third, and fourth culture generation from single colonies were injected in some instances. Injections were made of the broth culture or the

bacteria suspended in salt solution. In some instances cultures in ascites-tissue fluid were injected. The dose for the broth culture was usually from 0.5-3 c c for guinea-pigs and 2-5 c c for rabbits. The suspensions for injection were made so that 1 c c of the salt solution contained the growth from 15 c c of the broth culture, the dose of the suspension being 0.5-3 c c for guinea-pigs and 2-5 c c for rabbits. The injections in rabbits were made into the marginal ear-vein, those in the guinea-pig, dog, and cat into a leg-vein, the inoculations being made quite rapidly through a 21 or 23 gauge needle. A blood-agar plate culture was made of all suspensions immediately after injection to prove viability and kind of organism. Only animals from perfectly healthy stock were used. Young animals in each species were chiefly selected. The animals were examined two or more times daily for evidences of arthritis, myositis and other lesions, and for symptoms referable to the central nervous system. Chloroform was used to kill the animals for examination. Necropsies were made as soon as possible after death. Careful search was made for lesions at the point of injection, and of joints, muscles and nerves to make sure that the symptoms observed during life were not due to these causes. Cultures were made on blood-agar plates and in tall columns of dextrose broth (15 c c) as a routine from blood (0.1-0.5 c c) and the aspirated brain-substance containing more or less ventricular fluid (0.1-0.3 c c), and in numerous instances from other tissues.

The brain and cord were removed and placed into 10% formalin or into Kaiserling solution for 12-48 hours. Numerous cross sections at levels of about 3 mm. were then made in search of lesions. The portions put aside for sections for microscopic study consisted as far as possible of blocks showing gross lesions. The above solutions and Zenker's solution were used as fixing fluids. The tissues were embedded in paraffin and the sections stained with hematoxylin and eosin, with methylene blue and eosin and by the Gram-Weigert and Giemsa methods for bacteria.

ILLUSTRATIVE PROTOCOLS OF ANIMALS AFTER INTRAVENOUS INOCULATION

GROUP 1.—*Guinea-pigs.*—Guinea-pig 262, weight 200 gm., was injected Aug. 2, 1916, with 2 c c of ascites-dextrose broth culture of the tonsil from Case 695.

Aug. 3, the animal seemed well but there was undoubted weakness of the extensors of the right hind leg. The next day it was reinjected with washings from a scant growth on two blood-agar plates. Aug. 5, it was weak in the hind extremities and in the muscles of the back (Fig. 1). This was more pronounced the next day, but by Aug. 8 it had regained almost normal strength in the extremities and muscles of the back. The animal was chloroformed.

Cultures of the blood were sterile. No gross lesions were found and no cultures were made from the brain and cord. Microscopically, there were found thrombosis of the veins of the surface and interior of the lumbar cord, localized areas of hemorrhage in the cord, moderate degeneration of the ganglion cells and neurophagocytosis in areas. Diplococci were present in the areas of hemorrhage in the cord and those surrounding the spinal ganglia.

Guinea-pig 264, weight 250 gm., was injected Aug. 3, 1916, with 2 c c of ascites-dextrose broth culture (18 hours) of streptococci obtained from a single colony of blood-agar plate from the tonsil pus of Case 695. The next morning the animal appeared well. At 1 p. m. it had marked tremor and weakness of the left front leg. At 4:30 p. m. the weakness in the left front leg was increased, there was general tremor over the whole body; the animal was very excitable, and was moving about constantly. When the hind legs were held the tremor was constant, moderately coarse, and very marked. The left foreleg dragged with a complete toe-drop. The thorax was kept from the floor with difficulty, due to the weakness and there was rapid fatigue of the extensors of the left foreleg. The right foreleg was fairly normal. Following moderate exercise toe-drop developed; with more activity the entire leg dragged. While a moving picture was taken a similar condition gradually developed in the right hind leg so that finally the animal lay with both hind legs stretched out behind. With exhaustion the tremor became more marked, and twice there was a true convulsion of the left hind leg lasting from 30-40 seconds. Reflexes were not obtained. The tone of the muscles of the left foreleg was decreased. At 5:30 p. m. there was complete extensor paralysis of both the hind legs and the left foreleg. There was a repeated twitching of the left posterior hamstring muscles and sharp contractions of the diaphragm, resembling a hiccough. At 5:45 p. m. a general spastic condition with a tendency to retraction of the head developed and there were a number of general convulsions. Dyspnea was marked and the breathing was entirely diaphragmatic. Death occurred from respiratory failure at 6 p. m.

Examination showed a marked hyperemia of the extradural plexus, with softening and edema of the cord, and hemorrhages of the anterior surface of the medulla and pons. There were hemorrhages of the anterior root of the left third dorsal nerve with small hemorrhages in the left sciatic nerve, and edema of the legs. Cultures in blood-agar plates of the cervical cord, brain, lumbar cord, and muscles showed a moderate number of very fine, dry, slightly green-producing colonies of streptococci. Cultures from the blood, spinal fluid, kidney and liver were sterile. Numerous cross sections of the brain and cord, hardened in formalin, showed marked hyperemia of the vessels of the gray matter of the cord, together with a number of small hemorrhages, but no lesions in the brain. Microscopically, a mild meningitis and degenerative hemorrhagic lesions in the brain and spinal cord, and streptococci in the lesions were found.

Guinea-pig 266, weight 240 gm., was injected Aug. 4, 1916, at 9 a. m. with 2 c c of ascites plain broth culture (18 hours) from the tonsil of Case 695. The same day at 9:30 p. m. the animal appeared sick, uncomfortable, and irritable. The extensors of the hind legs were weak and walking was difficult. The hind legs slipped back and constant effort was made by the animal to get them under it. The muscle-tone of the hind legs was diminished. No resistance was felt in the extensors of the wrists and knees. Hiccough occurred at intervals. Aug. 5, the animal was found dead.

A number of hemorrhages had occurred in the stomach, cecum, papillary muscles of the left ventricle, and the liver, and a few in the lungs. There were edema and congestion of the pia, and hemorrhage and edema surrounding the dura and spinal ganglia. The lymph glands were hemorrhagic. There was

edema in the region of the solar plexus. No lesions were found in the nerve-trunks, joints or muscles. Cultures from the brain-substance showed green-producing streptococci, those from the blood showed a few colon bacilli but no streptococci. Microscopically, there were found a mild meningomyelitis with degeneration of ganglion cells and a moderate number of gram-positive diplo-cocci in the hemorrhagic areas surrounding the spinal ganglia.

Guinea-pig 270, weight 260 gm., was injected Aug. 4, 1916, at 9 a. m., with the growth from 30 c c of ascites plain dextrose broth culture from the tonsil of Case 695. At 9:30 p. m. the symptoms were almost exactly like those of Guinea-pig 266. The animal appeared sick, uncomfortable and was very irritable. There was weakness of the extensors of the thighs and legs. The hind legs could not be kept from slipping back, and it constantly made efforts to get them under the body. In walking it could not come up on its toes. There was occa-sional twitching of the muscles of the back and the hind legs and the latter tended to spread apart as though the adductors were weak. Walking required great effort. There was occasional hiccough. Aug. 5, the animal was found dead.

There were hemorrhages in the papillary muscles of the left ventricle, in the liver, stomach, cecum, and a few in the lungs. The mesenteric glands were hemorrhagic and there was marked edema in the retroperitoneal space and about the pancreas. The cord was very soft. There was marked congestion of the vessels of the pia, and hemorrhage and edema surrounding the cord and ganglia. Cultures showed from the brain a large number of streptococci, varying greatly in size, shape and grouping, and from the blood a smaller number together with colon bacilli. The microscopic sections showed focal hemorrhages and early infiltrative meningitis and myelitis, chiefly with round cells. Streptococci were demonstrated in the meninges and in the hemorrhages in the gray matter of the cord.

Guinea-pig 324, weight 100 gm., was injected Aug. 21, 1916, at 9 a. m., with 0.5 c c of ascites-dextrose broth culture inoculated with a single colony in ascites-dextrose-agar, originally isolated from the spinal cord of Case 707. At 5 p. m. there was marked tremor of the legs, and marked opisthotonos. The animal seldom moved the left hind leg, rarely extended the left hind leg or foreleg, and then weakly. It could not stand. The muscle-tone of the left hind leg was much below normal. At 9 p. m. the animal was found lying on its side with head retracted, and having convulsive spasms at intervals of from a few minutes to 10 minutes. There were coarse tremor and clonic spasms in the muscles of the extremities and of the eyelids. It was unable to stand and pawed the air with the legs, always less actively with the left hind leg. At 9:30 p. m. the condition was much the same. The temperature was 97.6 F. There were gen-eralized convulsions with the head markedly retracted and marked twitching of the muscles of the face. At 10 p. m. it was etherized.

The brain and cord were quite soft. The pia appeared opaque. The cere-brospinal fluid was clear. There was a moderate amount of clear fluid in the peritoneal cavity, and the mesenteric lymph glands were edematous and hemor-rhagic. There was marked edema of the retroperitoneum in the region of the splanchnic area and about the pancreas. No lesions were found in the viscera. Cultures from the blood showed a few, while the spinal fluid and the brain showed countless numbers of very fine, slightly green-producing colonies of streptococci. Cultures from the kidney were sterile. Microscopic sections showed chiefly meningitis with round cells predominating. There was marked conges-tion of the pia and the vessels of the gray matter, with slight perivascular infil-tration, degeneration of the nerve cells and areas of edema and hemorrhage in the gray matter, marked degeneration of the nerve cells, and neurophagocytosis.

Guinea-pig 337, weight 195 gm., was injected Aug. 23, 1916, with the growth from 3 c c of ascites-dextrose broth culture of streptococcus from the spinal cord of Guinea-pig 324. Aug. 25, the animal appeared well but showed weakness in the right hind leg. It was reinjected with ten times the original dose. Aug. 26, it appeared quite well but was weak in both hind legs, the weakness being more marked in the left. Aug. 27, the weakness had extended to the left front leg. The temperature was 104.6 F. Aug. 31, the weakness in the hind legs was still present. The animal was just able to get the hind legs under it but was scarcely able to walk. Both hind feet tended to turn outward, while the weakness in the left front leg was less marked. Sept. 2, the animal had grown very thin but was taking more food and its general condition was better. Weakness was most definite in the extensors of the left hind leg, and perhaps also in the adductors. There was no arthritis and a few steps could be taken slowly. Sept. 6, the condition was about the same. The left hind leg was very weak. The hind quarters frequently dropped to the left. There were contractures of the muscles and inability to extend completely the left knee and ankle-joints (Fig. 3). Sept. 7, at 4 p. m. the animal was found dead.

There was marked atrophy of the muscles of the hind legs out of proportion to the general wasting. The contents of the large and small bowel were fluid. There was an area of marked edema and hemorrhage in the sigmoid 12 cm. from the rectum, due to partial intussusception. There was edema in the retroperitoneum about the pancreas, and a few small hemorrhages in the cortex of the kidney. There were no lesions of the joints. The vessels of the pia were hyperemic, and the brain and cord were soft. Cultures from the brain, cervical cord and lumbar cord showed fine, dry, green-producing colonies of streptococci, those from the blood and liver remained sterile, and those from the kidney showed many green colonies of streptococci.

Guinea-pig 352, weight 165 gm., was injected Aug. 24, 1916, with 2 c c of ascites-fluid culture in the second generation from the spinal fluid of Guinea-pig 324. Aug. 25, the animal appeared quite well, but was spastic and weak in both rear and the left front extremities. It was reinjected with growth from 30 c c of ascites plain broth culture. Aug. 26, it appeared better, though weakness in the left hind leg was still noticeable. Aug. 28, it was found dead.

There were a few lesions in the muscles, a mild embolic nephritis, and a number of sharply circumscribed hemorrhages beneath the dura of the cord and the anterior surface of the medulla. Cultures from the brain showed an enormous number of fine dry, green colonies of streptococci, while those from the blood showed ten moist green colonies. Numerous cross sections of the brain and cord showed a number of localized hemorrhages in the brain. Microscopically, there were found a diffuse mixed-cell meningitis, hemorrhages, degeneration and slight infiltrative changes in the brain and cord, and a large number of streptococci in and adjacent to the areas of hemorrhage (Fig 8).

Guinea-pig 374, weight 190 gm., was injected Aug. 26, 1916, at 5 : 30 p. m., with the growth from 22.5 c c of ascites-dextrose broth culture of streptococcus from the brain substance of a cat injected intracerebrally with emulsion of the brain of Case 714. At 10 p. m. the animal seemed weak in the left hind leg. Aug. 27, at 9 a. m., it was weak in both hind legs, especially the right, carried its weight on the left side and often dropped quickly to the right. No difference in the strength in the forelegs could be made out. At 10 : 30 a. m. it was tremulous, the weakness in the hind legs was more marked and had extended to the right front leg; it was just able to walk. At 7 p. m. the hair was ruffled, the respirations were increased, the temperature was 101.8 F. and weakness in both front legs was evident. Aug. 28, it was found dead.

There were hemorrhages and edema surrounding the cord, and marked hyperemia of the vessels of the meninges and gray matter of the cord. Cultures from the brain substance in ascites-dextrose broth showed pure growth of the pleomorphic streptococcus. Microscopic sections showed marked hemorrhagic and infiltrative leptomeningitis, and marked disseminated infiltrative encephalo-myelitis, with perivascular infiltration, advanced nerve-cell changes, round-cell invasion, and a large number of streptococci in the infiltrated and hemorrhagic areas (Figs. 6 and 7).

Guinea-pig 384, weight 220 gm., was injected Aug. 27, 1916, with the growth from 10 c c of ascites-dextrose broth from streptococci from the brain substance of a guinea-pig injected intravenously with a culture from the tonsil in the second culture from Case 712. The animal seemed quite well until Aug. 30, when it was weak in the left foreleg, and both hind legs, was just able to walk and tended to fall to the left side. There was no swelling of the joints or at the point of injection. Oats were eaten and water drunk. It was unable to draw the hind legs under it (Fig 4). Sept. 2, the animal was very thin but ate well and the general condition was good. It walked a little and could pull its hind legs under it. There was marked weakness of the muscles of the back. When it was laid on one hip or the other with the front legs under the body, it could not raise its hind quarters. Abductor weakness was apparent in the left thigh, and more marked extensor weakness in the right hind leg. The extensors of the front legs were quite normal. Sept. 7, it ate well, appeared better generally, could get up when lying on either side though weakness was still most marked in the extensors of the right hind leg. Sept. 19, it was found dead; weight, 150 gm.

There was marked atrophy of the muscles of the hind extremities, especially the quadriceps, and marked contracture of the hamstrings on both sides. There were no gross lesions. Cultures were negative. Microscopic sections showed degenerative and infiltrative changes in the meninges and cord. A few diplococci were found in the meninges.

Guinea-pig 396, weight 170 gm., was injected Aug. 29, 1916, at 5:45 p. m., with growth from 15 c c of ascites plain broth of the streptococcus from the brain of Guinea-pig 374. Aug. 30, at 8 a. m., the animal was excitable, tremulous, and definitely weak in the left hind leg. At 5 p. m. it fell constantly to the left; both hind legs were weak, especially the extensors, and it was barely able to advance its legs forward under the body. It seemed in pain when handled. There was undoubted weakness in the left foreleg. Aug. 31, at 12 m. both hind legs were weak, especially the right, which it could not draw forward (Fig. 5). Sept. 2, the animal seemed much better, and got about well on three legs. The left hind leg was held up, suggesting arthritis, though this condition could not be made out. It was still irritable. There was marked extensor weakness of the left hind leg, but it could pull its legs under it. The right hind leg seemed normal. Sept. 5, it was found dead.

There was marked myocarditis, acute splenitis, arthritis of the left ankle, and marked softening of the brain and spinal cord. Cultures from the brain, spinal cord and blood showed streptococci. There was marked meningitis with exten-sion into the adjacent nerve-tissue. There were hemorrhages and degeneration of the brain and cord, and many diplococci in and adjacent to the lesions. The infiltrating cells were chiefly mononuclear.

Guinea-pig 422, weight 200 gm., was injected Sept. 2, 1916, with the growth from 15 c c of ascites-dextrose broth inoculated with the emulsion of the tonsil of Case 722. The next day the animal was very excitable, shaky and tremulous; it rushed blindly about the cage, and fell frequently, always to the left. The head was retracted. Both left legs were weak. There was purulent conjunctivitis in both eyes. Sept. 4, it was found dead.

Myocarditis, slight edema of the lungs, marked hemorrhage and edema surrounding the dura of the lower dorsal and lumbar cord and hyperemia of the vessels of the pia were found. Cultures on blood-agar plates from the blood were sterile, but from the lumbar cord and brain they showed a few fine green colonies of streptococci. Microscopically, there was found a predominating meningitis with mononuclear and polynuclear cells and slight infiltrative but marked degenerative lesions in the brain and cord. Streptococci were easily demonstrated in the areas showing lesions.

Guinea-pig 430, weight 180 gm., was injected Sept 5, 1916, with the growth from 30 c c of ascites-dextrose broth culture of small green-producing streptococcus from the tonsil in Case 722 in the third culture. The next day the animal apparently had a peculiar tic; the head was turned to the left and jerked constantly about 180 times per minute, the jaws working at the same rate. It was very irritable and excited, and was constantly on the move, turning to the left. Hay was taken in the mouth, but not swallowed. The front legs were fairly steady but the hind legs were weak and perhaps ataxic. The hind quarters frequently fell to the right, but an upright position could nearly always be regained. There was definite extensor weakness of the hind legs, especially the right; probably also abductor weakness. There was no spasticity and no nystagmus. Sept. 8, the weakness of the left hind extremity was more marked, the extensors were about the same, the animal walked weakly, and the hind quarters tended to drop to the left. There was prolapse of the rectum which was easily reduced. Oats were eaten. It was found dead the following day.

Necropsy showed hemorrhage and edema about the prolapsed portion of the rectum. There was hyperemia of the vessels of the pia and hemorrhage and edema surrounding the dura and cord; no lesions of the viscera. Cultures from the kidney, brain, cord, blood and liver showed colon bacilli, evidently due to infection from the rectum. Berkefeld N filtrates of the brain emulsion yielded pure culture of the pleomorphic streptococcus. Microscopically, lesions of the central nervous system were similar to those found in Guinea-pig 422.

GROUP 2.—*Rabbits, Dogs and Cats.*—Rabbit 962, weight 670 gm., was injected Aug. 20, 1916, with the growth from 30 c c of ascites-dextrose broth culture from the secretions in the throat and tonsil of Case 706. The following day the animal seemed quite well but was definitely weak in the hind extremities. Aug. 25, at noon, it was found lying on the right side with the head and eyes turned far to the right, and there was marked nystagmus. The right fore and hind legs were very weak, the left being less so and spastic. It was unable to stand and when placed on the left side it quickly flopped to the right, but when placed on the right side it could not get up. The muscle tone and reflexes on the right side were diminished. The left front leg was almost spastic. In the afternoon it was very weak, just able to walk. It drank water and ate oats. The following day the weakness was less marked and the temperature was 104 F. Aug. 28, it was found dead.

Vegetative, aortic and mural endocarditis, a few embolic abscesses and infarcts in the kidneys, hemorrhagic edema surrounding the dura of the cord, subdural hemorrhages between the cerebellum and cerebrum, and on the anterior surface of the medulla, were found. The fluid in the anterior chamber of the right eye was cloudy. Cultures from the blood and kidney showed a few colonies of staphylococci. Those from the brain gave a pure growth of rather moist green colonies of streptococci. The microscopic sections showed slight infiltration of the meninges and the gray matter of the spinal cord, marked degeneration of nerve cells, especially in the anterior horns, and streptococci in the pia.

Rabbit 977, weight 420 gm., was injected Aug. 24, 1916, with 6.5 c c of the second culture-generation of the spinal fluid of Guinea-pig 324. The following day the legs were weak, especially on the left side. If lying on the right side it could get up, but if on the left side it could not do so. It remained quiet unless started, when it would go a little way fairly normally and then become exhausted. Respirations were normal and it did not appear sick otherwise. It was chloroformed.

A moderate number of subperitoneal hemorrhages in the appendix and sigmoid, and a small amount of fluid blood in the peritoneal cavity were found. There were hemorrhages in the gallbladder and a large number of circumscribed submucous hemorrhages in the pyloric end of the stomach. There was marked edema surrounding the dura of the spinal cord, especially the cervical and lumbar enlargements. The cerebrospinal fluid was increased in amount and slightly blood-tinged. The vessels of the pia were markedly congested. There were a few small hemorrhages in the right sciatic nerve, in the left external popliteal space, and in both posterior tibial nerves; a few small hemorrhages and edema in the muscles about both hip joints, marked hemorrhage in one of the left gluteal nerves, and a few small hemorrhages in the nerve trunks of the forelegs. Cultures from the blood, brain and peritoneal fluid showed pure cultures of the green-producing streptococcus.

Rabbits 980 and 981, weights 410 gm. and 350 gm., were injected Aug. 25, 1916, with 3 c c, respectively, of broth culture from the tonsil emulsion from Case 714. Eight days later Rabbit 980 developed a peculiar gait caused by marked weakness of the adductors of the left hind leg. Eleven days after injection the extensors of both hind legs were weak. Gradual recovery took place during the following week. It died on the 20th day. Rabbit 981 remained apparently well until the 19th day when it was found lying on its side, moving the head very little and the extremities not at all. Respirations were slow, laborious and were almost wholly diaphragmatic. The animal died, apparently of respiratory failure, on the same day.

In both animals there was marked increase of cerebrospinal fluid; hemorrhage and edema surrounding the dura, congestion of the vessels of the pia, but no lesions of viscera, joints, or muscles and nerve trunks. Cultures of Rabbit 980 from the brain, lumbar cord, blood, liver and kidney were negative. Of Rabbit 981, cultures on blood-agar plates of the spinal fluid, brain, lumbar cord, liver, and blood were negative, but cultures in ascites-dextrose broth of the brain and blood yielded pure culture of a markedly pleomorphic streptococcus. Microscopic sections of both showed a slightly infiltrative meningomyelitis. At the medullary, cervical, and lumbar levels the meninges showed hyperemia, hemorrhages, a few round cells, and an occasional diplococcus. Lesions in the gray matter were more evident and consisted of hyperemia, numerous focal hemorrhages, slight adventitial and diffuse infiltration with round cells, increase of the neuroglial cells, nerve cell degeneration, satellitosis, neurophagocytosis and streptococci and small round cells (Fig. 18). The white matter showed slight gliosis and similar lesions were found in the spinal ganglia. The brain showed no infiltration.

Rabbit 982, weight 260 gm., was injected Aug. 25, 1916, with the growth from 22.5 c c of the culture from the tonsil emulsion of Case 714. Aug. 27, there was lacrimation and circumcorneal injection. The fluid in the anterior chamber of the eye was cloudy. Aug. 28, 8 a. m., there was a coarse tremor of the whole body, marked weakness of the front legs, but the animal was able to walk. At noon the tremor was much the same, the weakness in the frontlegs, especially the right, had increased. At 5:30 p. m. the right front leg was flaccid; the left was very weak, but could be used a little. It could not lift the thorax, head or

hips off the floor of the cage. Respirations were entirely diaphragmatic. There was considerable weakness but no paralysis of the hind legs. The tremor had disappeared. At 10 p. m. it was found dead.

The pericardial sac contained a small amount of turbid fluid and fibrin flakes. There were a few white muscle lesions in the inner aspects of the right thigh and about the right hip. The fluid in the anterior chamber of both eyes was turbid. There were beginning tricuspid endocarditis, marked edema of the dura and surrounding tissues, hemorrhage surrounding the spinal nerve roots, marked hyperemia of the vessels of the pia and hemorrhages in the gray matter of the cord. Cultures from the brain, blood and anterior chamber of the eye showed pure growths of greenproducing streptococci resembling pneumococci, while cultures from the pericardial fluid showed pure growth of hemolyzing streptococci. Microscopic sections showed a moderate, chiefly mononuclear, leptomeningitis more marked over the brain, and degeneration and gliosis in the brain and cord, with slight infiltration of the gasserian ganglion. Small hemorrhages were seen in the brain and medulla only. The choroid plexus showed vascular dilatation, edema and hemorrhage. Diplococci were found in the lesions.

Rabbit 985, weight 630 gm., was injected Aug. 26, 1916, with 4 c c of culture of the brain of Rabbit 979 which was injected intracerebrally with the emulsion of tonsil of Case 714. The second day at 4 p. m. the animal was very excitable. When put on the floor it rushed about toward the right in three-foot circles. After a minute or so the right front and hind legs gave out, it fell and was unable to get up again. Respirations were rapid. After resting it was evident that use of the abductors and muscles of the right hind leg was completely gone so that the leg remained crossed under it. The extensor muscles of the right hind leg were so weak they could not be brought forward. The left hind leg also showed abductor weakness and the leg crossed under it, but to a less extent than the corresponding right. The right foreleg was weak, and after exercise lay out motionless perpendicular to the body. The left foreleg was also weak but could be used. The head tended to fall to the right. Two convulsions occurred after which the respirations became very rapid, irregular and gasping. At 5 p. m. there was complete flaccid paralysis of the right extremities in addition to the abductor weakness now more marked in the left hind leg. No reflexes were obtained. The animal died of respiratory failure at 5: 10 p. m., the heart continuing to beat for 3 minutes.

The viscera were negative except for some small subdural hemorrhages in the insertions of the muscles about the knees and hips, probably traumatic. There was marked extradural hemorrhage, edema in the first and second cervical segments and about the medulla, and subdural hemorrhage on the posterior aspect of the second cervical and third dorsal segments. The brain was generally edematous, and there was hemorrhage of the sheath of the left optic nerve, symmetrical hemorrhage at the highest portions of the cerebellar hemispheres, and smaller hemorrhages in the other portions of the cerebellum and pons. Smears of the surface of the brain-substance showed many tiny gram-positive diplococci. The blood-agar plate cultures from the blood were sterile; those from the spinal fluid and brain substance showed a great many fine, dry, green colonies of streptococci in pure culture. Microscopic sections showed hemorrhagic, infiltrative and disseminated meningomyelo-encephalitis extending throughout the brain and cord, more marked in the cerebellar, cervical and lumbar regions. There was diffuse nerve-cell degeneration and neurophagocytosis. The infiltrating cells were mononuclear (Fig. 17). Streptococci were demonstrated in the lesions in large numbers in the depth of the tissues, while in the meninges a moderate number of leukocytes were found.

Rabbit 999, weight 500 gm., was injected Aug. 30 and 31, 1916, with 3 c c and 5 c c, respectively, of the culture from the brain of Rabbit 978, which was injected intracerebrally with an emulsion of the brain of Case 714. Sept. 2, there was marked ataxia, the animal could not walk and when standing swayed as though dizzy and fell repeatedly to the left. It was excitable, had a coarse tremor of the head and forelegs, and a coarse lateral nystagmus with the quick component to the right. There was marked weakness of the extensors of the left hind leg; and greatly exaggerated knee jerks. The right side was spastic with frequent coarse jerks of the legs and at times a slight retraction of the head. The following day there was almost complete flaccid paralysis of both hind legs, marked weakness of the right foreleg, frequent general convulsions, and retraction of the head between spasms, coarse twitchings of the right front, and to a less degree, of the right hind leg. The nystagmus was still present, the knee jerks were absent. It was able to keep upright on its forelegs spread widely apart; the hind legs remained stretched out in complete extension. The ataxia had disappeared except for a slight unsteadiness of the head. Food was taken, but an attempt to eat solid food (carrot) brought on convulsions (Fig. 11). Sept. 4, the animal was found dead.

There were marked hemorrhage and edema surrounding the dura, especially in the lumbar and cervical regions, marked congestion of the pia, subpial hemorrhages near the median fissure in the right hemisphere, hemorrhage in the pia over the anterior surface of the medulla and pons, and one large hemorrhage in the substance of the pons and medulla near the median line. A slight arthritis of the left knee, but no lesions of the viscera except one hemorrhage in the pyloric end of the stomach were found. Cultures of the blood were sterile, while those from the lumbar cord and brain showed pure growths of fine dry, green colonies of streptococci. Microscopic sections showed infiltrative mononuclear meningitis with extension along the vascular adventitia into the adjacent nerve tissue, focal hemorrhages, round-cell infiltration and nerve-cell degeneration, particularly in the gray matter of the cord. Streptococci were numerous in the pia but were difficult to demonstrate in the nerve tissue.

Rabbit 1001, weight 450 gm., was injected Aug. 30 and 31, 1916, with the growth from 7.5 c c and 15 c c,respectively, of the culture from the brain of Rabbit 978. Sept. 3, the animal appeared sick and coughed; there was no lameness. The following day there was undoubted weakness in the front legs, more marked in the right than in the left. Sept. 6, the hind legs were rather clumsy and the knee jerks appeared exaggerated. Sept. 12, it was found dead.

No lesions were found except hyperemia of the vessels of the pia and a few hemorrhages in the gallbladder. Cultures from the blood were sterile; those from the brain showed a great many fine dry, green colonies of streptococci in pure culture. Microscopic sections of the brain, medulla and cord showed hyperemia and hemorrhages, in which streptococci of varying size were numerous. There were circumscribed areas of round-cell infiltration, often surrounding the blood vessels (Figs. 15 and 16). It was extremely difficult to find streptococci in the areas of round-cell infiltration. In some instances the disseminated areas of infiltration were more widespread and tended to be confluent, giving a picture of infiltration not unlike that in acute poliomyelitis in man and monkey.

Rabbit 1012, weight 330 gm., was injected Sept. 4, 1916, with the growth from 15 c c of the broth culture from a single colony of the tonsil emulsion in Case 722. Sept. 6, it was found dead.

There were marked softening of the brain and cord, marked congestion of the vessels of the pia and extradural plexus, and hemorrhages surrounding the cord. Cultures of blood and brain showed pure growths of fine, dry, green

colonies of streptococci, about ten times as many in the brain as in the blood. Microscopic sections showed beginning meningitis with numerous streptococci in the pia, marked degeneration in the nerve cells in the cord, and practically no cellular infiltration.

Rabbit 1017, weight 220 gm., was injected Sept. 5, 1916, with the growth from 15 c c of culture from the brain of Rabbit 999. Sept. 7, the animal was very unsteady and weak; there was constant tremor of the head and it was just able to stand. On the following day the power in the muscles of the front legs and the left side of the neck was gone. It could pull the hind legs under it, but the front legs were spread widely, and it could not bring the thorax off the floor (Fig 12). There was no nystagmus and no reflexes were obtained. It died at 9 : 30 a. m.

Cultures from the blood showed a few, from the brain innumerable, numbers of fine, dry, green colonies of streptococci; in those from the kidney there were many green colonies of streptococci, while in those from the liver and joints there was no growth. Microscopic sections showed diffuse hemorrhagic infiltrative meningitis extending along the central canal, infiltrative disseminations and hemorrhages, more marked in the brain and medulla, but progressively less marked in the spinal cord. Infiltration was predominatingly mononuclear and was especially noticeable in the pia and around the central canal (Fig. 13). Diplococci were found in the lesions in brain, spinal cord and meninges. They were most numerous surrounding blood vessels in the meninges and difficult to demonstrate in the areas of round-cell infiltration in the cord.

Rabbit 1032, weight 400 gm., was injected Sept. 12, 1916, with the growth from 15 c c of the culture from the brain of Monkey 10 which was injected with culture from the brain of Rabbit 1017. This strain from Case 714 was in the fifth animal passage. Sept. 14, the animal was found dead.

There were a few hemorrhages in the right knee joint, and the joint fluid was turbid. There were hemorrhage and infiltration of the muscles of the anterior surface of both tibias. There were numerous, very small punctate hemorrhages in the subcutaneous tissue, marked edema and hemorrhage in the lungs, with beginning bronchopneumonia, many small hemorrhages in the kidneys, and cloudy swelling of the viscera. There was hemorrhagic edema surrounding the cerebellum. The cerebrospinal fluid was turbid. There were localized hemorrhages under the dura in the lumbar region, and edema surrounding the cord and dura along the nerve roots and ganglia. Blood-agar plate culture from the blood and spinal fluid were negative. Those from the kidney showed countless fine, dry, green streptococci and those from the liver a moderate number of similar colonies. Microscopic sections revealed a large hemorrhage in the left cerebellar hemisphere and a moderate number of small hemorrhages in the medulla and cord, which gave the picture of a mild meningomyelitis. The infiltrating cells in the pia consisted of about equal numbers of round cells and leukocytes. Streptococci were present in enormous numbers surrounding blood vessels, especially in the pia and the more superficial vessels in the cord, and in localized areas showing hemorrhage or degeneration of nerve cells in the gray matter of the medulla and cord.

Dog 463, weight 4.5 kg., was injected Aug. 4, 1916, with the growth from 45 c c of broth culture from the tonsil of Case 695. On the following day at noon the animal was lame in the right hind leg (the injected leg). There was tremor in both hind legs, and apparently definite weakness. At 6 p. m. the muscular tremor had extended to the right front leg, and at 9 p. m. it was unable to stand, and lay on its side with head retracted and extremities held stiff. There was no swelling of the joints. Aug. 6, it was found dead.

There was marked cellulitis at the point of injection. There were hemorrhages in the lungs, gallbladder and liver, and marked congestion of the vessels of the cord and brain. The meningeal fluid was turbid, the pia over the brain was opaque, and the brain was edematous and soft. There were hemorrhagic spots in the anterior horns of the cervical and lumbar cords and smears from the meningeal fluid showed a few gram-positive diplococci. Cultures from the meningeal fluid and blood showed what appeared to be pure culture of pneumococci. Microscopic sections showed extensive meningitis and disseminated areas of infiltrative and perivascular infiltration principally with mononuclear cells. Hemorrhages and diffuse degenerative lesions were present in the brain and spinal cord and ganglia. Streptococci were demonstrated in the hemorrhagic areas and in the meninges in moderate numbers, and in smaller numbers in the areas of hemorrhage and round-cell infiltration in the substance of the cord.

Cat 11, weight 930 gm., was injected, Aug. 24, with the growth from 45 c c of tonsil pus from Case 712. Aug. 26, there was weakness in the hind legs, most marked on the right. The temperature was 104 F. Sept. 11, the animal was found dead; weight 690 gm.

		I	II	III	IV	V
			R998—16 c.c. V. +		P445—25 c.c. V. +	P450—15 c.c. V. 0
			R999—8 c.c. V. +	R1017—15 c.c. V. +	M10—1.5 c.c. C. +	
			R1000—30 c.c. V. +		M12—90 c.c. V. +	R1032—15 c.c. V. +
	Brain	R978—0.5 c.c. C. +	R1001—7.5 c.c. V. +			
			P406—2 c.c. V. 0			
		C13—0.5 c.c. C. +	P407—1.5 c.c. V. 0			
			P409—31 c.c. V. +			
	Dura	P389—30 c.c. V. +	P410—10 c.c. V. +			
Case 714		P390—30 c.c. V. +				
		P391—5 c.c. V. 0				
		P355—2.5 c.c. V. 0				
		P356—0.5 c.c. C. +				
		P360—19.5 c.c. V. 0				
		P361—24 c.c. V. +				
	Tonsil	P364—15 c.c. V. 0	R985—4 c.c. V. +			
		R979—0.5 c.c. C. +				
		R980—3 c.c. V. +	P382—8 c.c. V. +	P396—15 c.c. V. +		
		R981—3 c.c. V. +	P374—22.5 c.c. V. +			
		C12—0.5 c.c. C. +	P377—10 c.c. V. +	P397—7.5 c.c. V. +		
		R982—22.5 c.c. V. +	P386—15 c.c. V. +			

Text Fig. 1.—Chart showing results of injections of emulsions and cultures of the pleomorphic streptococcus from brain, edema fluid surrounding dura, and tonsil in a typical case of poliomyelitis before and after animal passage. V, intravenous inoculation; C, intracerebral inoculation; +, positive result; 0, negative result. All intracerebral injections were emulsions of brain or tonsil. All intravenous injections consisted of cultures or suspensions of cultures.

TABLE 1*

Elective Localization of Streptococci with Special Reference to Poliomyelitis

Diseases	Source of Streptococci	Strains	Animals	Percentage Incidence of Lesions																	
				Meninges	Brain	Spinal Cord	Posterior Roots of Ganglia	Nerves	Muscles	Joints	Skin	Stomach and Duodenum	Appendix	Intestines	Gall bladder	Liver	Kidneys	Lungs	Pericardium	Myocardium	Endocardium
Acute poliomyelitis....	Tonsil	22	123	41	19	46	12	4	16	15	2	13	2	5	2	2	2	11	5	7	7
Acute poliomyelitis....	Brain and cord	8	18	38	17	38	17	2	8	8	2	6	2	4	0	4	8	6	0	4	2
Transverse myelitis....	Tonsil	1	21	50	24	67	0	5	24	11	5	24	5	33	0	0	10	10	10	14	14
Neuralgia..........	Tonsil	4	18	22	0	6	83	28	33	11	28	6	0	0	6	6	6	33	0	6	22
Multiple neuritis........	Tonsil	1	19	0	5	5	0	79	27	22	0	11	11	11	5	0	16	32	16	11	5
Miscellaneous non-specific strains......	Tonsil	85	366					0	12	27	2	20	5	8	11	0	9	11	2	10	14

* This table shows the total incidence of lesions in various organs in animals following intravenous injection of streptococci. The organs in which lesions were not found or only rarely are omitted. The symptoms referable to the nervous system in the 366 animals injected with strains from miscellaneous sources were so rare that the nervous system was not examined as a routine. The figures indicating localization are given in percentages and hence are directly comparable.

There were no lesions of the viscera but edema and cloudiness of the meninges and a large amount of turbid cerebrospinal fluid. Blood-agar plate cultures from the kidney, liver and blood were negative, those from the spinal fluid, brain and lumbar cord contained countless numbers of fine, dry, green colonies of streptococci. Sections of the central nervous system showed definite mixed-cell meningitis degenerations and hemorrhages in the brain and cord, and streptococci.

EXPERIMENTS ON INTRACEREBRAL INOCULATIONS

It was thought advisable to compare the results of intracerebral injections with intravenous injections. Hence, young guinea-pigs, rabbits and cats were injected intracerebrally with from 0.25 c c–1 c c of suspensions of pus from tonsils, and of emulsions of brain from human poliomyelitis and from animals showing paralysis, and filtrates of emulsions and cultures.

Emulsions from the tonsil of 6 cases of human poliomyelitis were injected altogether into 3 rabbits, 5 guinea-pigs and 4 kittens. Seven of the 12 animals developed flaccid paralysis usually complicated with symptoms of meningitis. In 9 the blood was sterile or nearly sterile, while the brain and spinal fluid contained many streptococci. In all, the green-producing streptococcus predominated, and in all but three instances it was isolated in pure culture (Rabbit 929 and Guinea-pig 433). Meningitis was usually the cause of death and the chief finding at necropsy. Recovery occurred in some instances (Rabbit 929).

Emulsions of the brain and cord from 6 human cases and from 4 animals showing flaccid paralysis were injected into 14 guinea pigs, 11 rabbits and 4 kittens. Flaccid paralysis developed in 8 animals; in 7 the pleomorphic streptococcus was isolated in pure culture from the nervous system, usually in large numbers, but not from the blood (Rabbits 978 and 1018). The other animals showed no symptoms. Ten died within 24 hours, most of them apparently from mechanical and toxic effects, the cultures being negative. Fourteen recovered completely, at no time showing symptoms. Five were chloroformed or died from 2–6 days after injection. The emulsions of the brain and cord from 3 human cases gave positive results, while the remaining 3 gave negative results.

Owing to the marked variations in size of the streptococcus having affinity for the nervous system, some forms being fully as small as the globoid bodies, filtrates of emulsion and cultures were used for injection. The first filtrate was cultured and injected Aug. 26, 1916. Berkefeld filter N Berkefeld filter V, and a dense porcelain candle were used. The latter is the type of filter which was used as a control in elective localization studies. Altogether 8 filtrates were made from emulsions

of brain and cord of animals showing flaccid paralysis. These were injected into 11 guinea-pigs, 12 rabbits and 2 kittens. Eight of the animals died within 24 hours, usually without paralytic symptoms, the blood and brain being sterile. Four died within 4 days, 3 showing streptococci in the brain but not in the blood. Thirteen remained well and were anesthetized a long time after injection.

In three instances parallel filtrates were made through a Berkefeld N and porcelain filters. Cultures and injections of the latter were negative. Two of the former yielded the pleomorphic streptococcus in pure form on culture. Injection of one of these was followed by paralytic symptoms (Rabbit 1023), the other rabbit showed streptococci in the brain but not in the blood. One other Berkefeld filtrate gave positive results in a rabbit but the cultures remained sterile. Four Berkefeld N filtrates were sterile and the results in animals were entirely negative. The lesions observed in these animals following intracerebral injection consisted chiefly of a meningitis with more or less infiltration and degeneration of the nerve cells in the brain and cord. In the animals that died early the meningitis was chiefly polynuclear; in the animals that lived for some time is was mononuclear. The infiltration was usually most marked around the blood vessels, around the central canal and in the anterior horns. Degeneration of the nerve cells in the medulla and anterior horns, more especially of the cervical cord, was noted. These lesions following intracerebral inoculation resembled very closely those described by Rosenau and Havens following successive injections of virus in rabbits.

ILLUSTRATIVE PROTOCOLS FOLLOWING INTRACEREBRAL INOCULATION

Rabbit 929, weight 660 gm., was injected July 21, 1916, with 1 c c of the emulsion in salt solution of the pus in Case 686. July 22, it appeared quite well, but was spastic and the knee jerks were exaggerated. July 24, it stood with head markedly retracted. The eyes were more prominent than normal. When made to walk it appeared awkward and unsteady and the right hind leg was thrown out. It became tired in a few minutes and the right hind leg was markedly weak, gave way and dragged. The right foreleg also was weak. The rabbit tended to fall to the right. The muscle tone in the hind legs and right front leg was low. Spasticity had disappeared and no reflexes could be obtained. July 25, it was lame in the left hind leg, the knee joint was swollen, but the right leg appeared stronger. Aug. 12, it had apparently regained full power in the extremities and appeared well. It was chloroformed.

The blood cultures were sterile, those from the brain yielded a pure culture of a streptococcus varying markedly in size, some forms resembling "globoids."

Guinea-pig 433, weight 200 gm., was injected Sept. 6, 1916, at 7:30 p. m. with 0.25 c c of the emulsion of the tonsil from Case 724. At 10 p. m. there was a fine tremor all over the body. It was excitable, acted strangely, pushed its head violently against the floor of the cage. There was some difficulty in walking, the hind legs tending to extend, and the tremor was much worse on attempts to

walk. At 10:20 p. m. it was found pushing violently with its nose against the dish containing oats. When freed from this position it ran its nose forcibly into the side of the cage and attempted constantly to bury it deeply in the hay. Sept. 7, it was found dead.

There was edema of the lungs, marked hemorrhage surrounding the dura, especially in the cervical region and cerebellum and medulla. There was no hemorrhage at the point of injection. Cultures from the blood were sterile, while those from the blood and brain showed the pleomorphic streptococcus in pure culture.

Rabbit 978, weight 480 gm., was injected Aug. 24, 1916, with 0.75 c c of the emulsion of the brain of Case 714 (Fig. 1). Aug. 27, there was no loss of power of the extremities, but the animal acted peculiarly with a tendency to lie down and stretch out the hind legs after it had been made to walk. Aug. 28 at noon, there was very marked general weakness. The extensors of the left foreleg were completely gone; those of the right were weak. The neck muscles were very weak; the head was not held up and usually fell to the left. The hind legs were generally weak, the right more marked, but it could pull them under it when extended. The respirations were slow and shallow; no motion of the ribs could be made out. It died at 1 p. m.

There were superficial recent hemorrhages in the mucous membrane of the stomach, and hyperemia and hemorrhage surrounding the dura in the cervical region. The brain showed no gross lesions. Cultures from the blood were sterile, those from the brain showed the pleomorphic streptococcus in large numbers. Microscopic sections showed the picture of a leptomeningo-encephalitis, the predominating infiltrating cell being mononuclear.

Rabbit 1018, weight 580 gm., was injected Sept. 6, 1916, with 0.5 c c of the emulsion of the brain in Case 724. Sept. 11 there was double foot drop behind. It could walk a few steps very awkwardly, dragging the toes. The legs spread apart as if there was adductor weakness. When the hind legs were pulled out back it got them under by pushing the body back with the front legs. The animal appeared in good general condition. Sept. 12, it moved about much better, though the left hind leg was undoubtedly weak. Sept. 15, it was found dead.

There was marked edema surrounding the dura of the cord; no apparent lesion at the point of injection. There were marked congestion of the vessels of the meninges, edema surrounding the medulla and cerebellum and no lesions of the viscera. Cultures from the blood were negative, those from the brain showed countless numbers of green colonies of streptococci resembling pneumococci. There were hemorrhagic meningitis, marked round-cell infiltration of the choroid plexus and localized areas of round-cell infiltration around the vessels of the brain, pons, and medulla (Fig. 19).

Rabbit 993,* weight 610 gm., was injected Aug. 30, 1916, with filtrate of ascites fluid culture of the brain of Rabbit 978. Aug. 31, the animal was found dead.

There were marked degeneration of the liver, cloudy swelling of the viscera, no hemorrhage at the point of injection, and edema of the brain and cord surrounding the dura. Cultures from the blood were sterile, while those from the brain yielded pure culture of the pleomorphic streptococcus.

Rabbit 1023, weight 670 gm., was injected Sept. 8, 1916, with 0.5 c c of a Berkefeld N filtrate of the emulsion of the brain of Rabbit 1017. Sept. 10, the head was markedly retracted, the animal moved constantly, could stand but was very ataxic when it attempted to walk. The hind legs were spread

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* The cultures of the filtrate injected were negative on blood agar but showed the pleomorphic streptococcus in deep cultures of ascites-dextrose tissue broth.

apart and there was marked adductor weakness, especially of the right leg. Respirations were rapid. Sept. 11, it was found dead.

The body was warm. There were numerous hemorrhages in the stomach and a few areas of infection in the left kidney; marked edema over the brain, turbid cerebrospinal fluid, and hemorrhages of the posterior aspect of the cerebellum. Cross sections of the medulla showed a number of hemorrhages in the gray matter and some hemorrhages in the cervical cord. Blood-agar plate cultures of the blood showed a few colon bacilli, but no streptococci. Cultures of the brain and lumbar cord showed countless numbers of the fine dry green colonies in pure culture, while those from the liver and kidney were negative.

SYMPTOMS

The symptoms observed varied greatly but were usually striking and easily differentiated from arthritis, myositis and from general weakness occurring in overwhelming infections. In many instances the animals appeared well, ate food normally, were active and seemed disturbed because of inability to use the paralyzed part. There were usually instability, restlessness and evidences of pain on handling, especially in the guinea-pigs (Pig 346). Tremor and twitchings of muscles of one or more extremities often preceded the development of flaccid paralysis (Pig 264). In some instances these premonitory symptoms were followed by recovery without development of paralysis. Increased reflexes followed by loss of reflexes and loss of muscle-tone were common findings. The paralytic symptoms varied from slight weakness, usually in extensor groups, to complete flaccid paralysis of one or more extremities.

Death from respiratory failure was the rule in the animals observed (Pigs 262 and 264, and Rabbits 981, 982, 985, etc.). Some died in convulsions. This was especially apt to occur when paralysis began in the fore extremities. The place of onset of the paralysis in some animals corresponded to that in the patient from whom the culture was obtained. This occurred too often to be accidental.

Flaccid paralysis was the striking symptom in many animals, but this was often associated early with more or less evidence of meningeal irritation (Pigs 262, 264, 266, 270, 352, 374, 384, 396, and Rabbits 962, 977, 980, 981, 982, 1001, 1017). In others the paralysis appeared to be associated with a definite meningitis (Pigs 234, 422, 430, Rabbit 985, and Dog 463). In a few cerebellar symptoms predominated (Rabbit 999). A number of guinea-pigs and rabbits and one cat showed symptoms suggesting mental derangement (Pigs 422, 430, 433, and Rabbit 985). The time of onset of symptoms was related both to the size of the dose and to the infecting qualities of the particular strain injected. Thus, in some instances the symptoms began late if the dose was small

(Rabbits 980 and 981) and early if the dose was large (Rabbit 982), while in others they began simultaneously and ran the same course even though the dose varied by the fifteenth multiple (Pigs 266 and 270). In some instances paralytic symptoms began as early as 4 hours, and often within 24 hours, following inoculation (Pigs 264, 266, 270, 324, and Rabbits 962, 977 and 985). The symptoms were prone to be of an explosive character when the period of incubation was long (Rabbits 980 and 981). This finding is in accord with a similar observation made by Rosenau and Havens following injection of virus.

GROSS AND MICROSCOPIC CHANGES

The gross pathologic findings consisted of hyperemia of the vessels of the meninges and an increased amount of cerebrospinal fluid, usually clear, sometimes slightly turbid, and in a few instances markedly turbid. The anterior extradural plexus, particularly at the lumbar and cervical enlargements of the cord, showed extreme engorgement. The anterior surface of the dura was often hemorrhagic and edematous. Hemorrhage and edema around nerve-roots and spinal ganglia were a common finding. The pia in some instances appeared opaque but was usually moist and slimy. Hyperemia, edema and focal hemorrhages in the gray matter of brain, medulla and cord, chiefly in the anterior horns, were frequently noted.

The lymph glands, particularly those in the mesentery and retroperitoneum, were often swollen, edematous and hemorrhagic. The lymphoid elements in the intestines at times appeared swollen. There was often marked edema in the retroperitoneum in and about the solar plexus and pancreas in guinea-pigs (Pigs 266, 270, 324, and 337). This has never been seen following intravenous injection of streptococci from sources other than poliomyelitis. The spleen rarely showed enlargement. The lesions in other organs were conspicuously rare and when they did occur were relatively slight.

Microscopically, the meninges and nerve tissue showed a variable vascular reaction ranging from slight hyperemia to marked engorgement. In many cases this was accompanied by perivascular and interstitial hemorrhages, more marked in the gray matter. Infiltration with small round cells (Fig. 13) occurred in the pial membrane and in the adventitia about the blood vessels (Figs. 6, 15, 16, and 19). In other cases the infiltration was diffuse over wide areas in the interstitial tissues, or more often in circumscribed areas either around a small vascular focus or circumscribing a central point of cellular degeneration

(Figs. 13, 14, 17, and 19). In places these islands tended to be confluent. Generally the lesions varied from the larger and more discrete areas to the smaller and more confluent areas resembling the finely disseminated coalescing lesions in typical poliomyelitis. Where they were more extensive a central focus of hemorrhage, or, more often, an area of dense cellular infiltration with disintegrated stroma was surrounded by a wide zone of mononuclear infiltration both about the blood vessels and in the intervening tissues. A section through the peripheral zone of such an area gave a picture microscopically not unlike that in the diffuse infiltration of an acute poliomyelitis in man. Until serial sections were studied to determine the presence or absence of a central focus, such an infiltration could not be accurately interpreted. Some animals showed adventitial tissue and diffuse interstitial cellular infiltration with no evident central focus (Figs 13 and 14). In a few animals thrombosis of the vessels of the meninges and cord were found with little or no perivascular infiltration.

Cytologists do not yet agree regarding the origin of the infiltrating round cells, hence no special attempt was made to identify them. Various types resembling lymphocytes, proliferated endothelial cells and glial cells took part in the reaction. Leukocytic infiltration was relatively slight. Even in animals showing meningitis the infiltration was usually predominatingly mononuclear. The infiltrative lesions in the depths of the brain and cord at times contained only mononuclear cells when meninges showed both (Rabbits 985 and 1017, Fig. 13). Leukocytic infiltration was more often prominent in experiments of short duration following injection of large doses (Rabbits 980, 981 and 982), and particularly after injection of these strains rendered more virulent by repeated animal passage (Rabbit 1032). The nerve cells often showed swelling, granular staining, and in advanced stages they stained poorly or not at all. In many instances small round cells, sometimes recognized as glial cells, collected around degenerated cells as satellites, but marked neurophagocytosis was not common.

Streptococci were found in or adjacent to the lesions in the central nervous system in all animals that showed symptoms not too long preceding the time of death. In the animals in which the experiment was of short duration and the lesions were acute, the demonstration of streptococci was relatively easy. The streptococci here were most numerous in the perivascular lymph spaces and other areas where infiltrative lesions more often became marked in later stages. In the perivascular areas they were of uniform size, resembling pneumococci, while in the infiltrative areas great variation in size were

noted, some diplococi being fully as small as the "globoid" organism described by Flexner and Noguchi. Streptococci were found in the edematous and hemorrhagic areas surrounding the dura, in the pia, in the adventitial lymph spaces surrounding dilated blood vessels (Fig 9), in edematous and hemorrhagic areas in the brain and cord (Figs. 8, 18, and 20) and in areas of perivascular and interstitial round-cell infiltrations (Fig. 7). In the latter it is often exceedingly difficult to find bacteria. In a few animals that had recovered completely from paralysis, streptococci could not be demonstrated. The tissues showing no lesions were quite free from streptococci even in experiments of short duration and where streptococci were demonstrable in the lesions that were present.

THE RESULTS

The results of the injection of cultures from the tonsils or throat of 22 cases and those from the brain and cord in 8 cases were studied in detail.*

Altogether 171 animals were injected intravenously; young guinea-pigs, rabbits, dogs, cats, and monkeys; 123 were injected with tonsil strains and 48 with brain and cord strains. The tonsil strains were injected into 75 guinea-pigs, 36 rabbits, 10 dogs, 1 cat, and 1 monkey; the brain and cord strains into 25 guinea-pigs, 19 rabbits, 1 dog, 1 cat, and 2 monkeys.

Positive results were obtained with cultures from tonsils made on repeated occasions, in one case on four occasions, and with cultures from different parts of the central nervous system (Text Fig. 1). In one instance completely negative results followed the injection of a pneumococcus from the consolidated lung when the streptococcus from the tonsil and the brain and cord from the same patient produced paralysis. The affinity for the central nervous system at the time of injection varied greatly in different strains. Approximately 70% of the animals were inoculated with the first culture generation, 25% with the second, and 5% with the third or fourth. When platings were made, subcultures for injection were made as soon as colonies became recognizable. Twenty-five per cent. of the rabbits weighed less than 500 gm., 55% between 500 and 1,000 gm., and only 20% above 1,000 gm. Fifty per cent of the guinea-pigs weighed less than 200 gm., 40% between 200 and 300 gm., and only 10% above 300 gm. The average weight of guinea-pigs showing positive results was about 200 gm., of rabbits about 600 gm. The average weight of guinea-pigs

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* For methods and results of cultures see Reference 48 and the paper in this series on the etiology of poliomyelitis.

and rabbits with negative results was about 300 gm. and 1,200 gm., respectively.

The diagnosis of localization in the central nervous system was based in the order of importance on four main findings: (1) Microscopic lesions of the central nervous system; (2) streptococci in or adjacent to these lesions; (3) positive cultures from the central nervous system when blood or other tissues were sterile or when they contained comparatively few bacteria, and (4) meningitic or paralytic symptoms.

In 40% of the animals, paralytic symptoms were noted during life. In approximately 70% of the animals in both series two or three of the diagnostic factors were present, leaving no reasonable doubt of the specific localization. In 15% following injection of strains from tonsils, and in 20% following injection of strains from brain and spinal cord, all the diagnostic factors were present and the specific localization proved beyond doubt.

Seventy-three of the 171 animals were chloroformed for examination after definite symptoms were established; 98 died. The mortality rate in rabbits was considerably higher than that in guinea-pigs. In both species it was somewhat higher following the injection of tonsil strains than with brain and cord strains. The total percentage incidence of lesions in various organs following injection of the poliomyelitis strains together with that of a number of other strains is given in Table 1. The tendency of the strains from poliomyelitis and transverse myelitis to produce lesions in the central nervous system was striking. The occurrence of lesions in other organs was comparatively rare following injection of the poliomyelitis strains, but occurred more often following injection of tonsil strains than following the injection of brain and cord strains. Lesions outside the central nervous system following injection of cultures from tonsils were found to be due in some instances to nonspecific strains of streptococci or staphylococci (Rabbits 962 and 982). Unless the specific affinity was marked on isolation, aerobic cultivation for several generations was sufficient to destroy the elective localizing power. Small doses after aerobic cultivation produced no lesions whatever; large doses produced slight lesions in stomach, kidney or joints with or without focal lesions in the central nervous system. Moreover, on repeated animal passages, even when cultures for succeeding injections were made from the brain or spinal fluid, the specific localizing power gradually disappeared. The range of localization and the degree of lesions became greater as virulence increased. Hemorrhages and edema of the lung, hemor-

rhages of stomach, suppurative arthritis and suppurative focal lesions in the brain and cord, and purulent meningitis were now more likely to occur (Rabbit 1032).

The incidence of localization before and after successive animal passages of the strain from the brain, from the edematous fluid surrounding the dura, and from the tonsil of Case 714, is illustrated in text Figure 1. Sixteen animals were injected intravenously with brain and dura strains and fourteen with the tonsil strain. Of the former twelve (75%), of the latter eleven (71%), showed undoubted symptoms or symptoms referable to lesions of the central nervous system. Some of the negative results were apparently due to the smallness of the dose (Pigs 391, 406, 407 and 955). Positive results were obtained when the dose varied from the second to the seventh multiple (Rabbits 998, 999, 980, 981 and 982). The strains isolated from many of these animals were cross-agglutinated specifically by the serum from a horse immunized with human strains and another horse immunized with monkey strains.[47] The serum from persons and monkeys which had recovered from poliomyelitic attacks had a similar but less marked agglutinating power.[47]

Cultures from the brain and cord, and blood of the animals were made routinely and from various other tissues in many instances. In most animals showing positive symptoms the brain and cord showed pure cultures of streptococcus, often in large numbers when the blood and other tissues (with the exception of the kidney) contained few or none (Pigs 264, 266, 270, 324, 352, Rabbits 962, 985, 999, 1001, 1012, and 1017). The importance of using partial oxygen-tension cultures (tall columns of dextrose or ascites-dextrose broth) in this work, aside from differences in the mediums, was shown by the fact that aerobic blood-agar plates from brain and cord usually remained sterile when the deep cultures in the broth were positive (Rabbits 980 and 981) unless the number of bacteria was large, then both gave positive results.

The number of streptococci isolated from brain and spinal fluid was, as a rule, definitely related to the duration of the experiment and sometimes to the symptoms referable to the central nervous system. This is in accord with the microscopic findings. The blood of the animals injected with the strains, as isolated, almost never showed large numbers of streptococci even in those that died within 24 or 48 hours after inoculation.

Control cultures, using the same technic, from brain and spinal fluid and blood were made in 35 animals (29 guinea-pigs, 4 rabbits

and 2 dogs) which were killed some months after inoculation. These had been injected either with small doses of the characteristic streptococcus, with hemolyzing streptococci from tonsils or with mixtures of bacteria from tonsils some time after recovery from the attack. In these streptococci were not isolated in a single instance. Cultures of tissues were made, moreover, in 40 uninoculated animals as controls; 33 of these were well and were killed for examination, and 7 died from unknown cause. One of the former and three of the latter showed a few colonies of green-producing streptococci in small numbers in the cultures from brain and spinal fluid, and in two of the latter indifferent streptococci were isolated from the blood and brain. Colon bacilli were isolated from blood and brain in a few that had died, but not from those killed immediately before examination.

DISCUSSION

Following the discovery that the virus of poliomyelitis is filtrable, ordinary bacteria were considered of little or no etiologic importance in this disease, until the demonstration recently that the tonsils and infected tissues in acute poliomyelitis contained streptococci which, when injected into animals, tended to localize in the central nervous system. Bull studied streptococci from tonsils in poliomyelitis and from the tonsils and teeth of persons who were not suffering from poliomyelitis, nor in immediate contact with the disease, but who resided in the midst of a well-spread epidemic. It is to be noted that his technic, although purported to be done "in the manner described by these authors," really does not fulfill the conditions emphasized as essential in the study of elective localization in poliomyelitis. Young animals must be selected. Only adult guinea-pigs and rabbits were used in Bull's experiments. Twelve guinea-pigs weighing from 450-500 gm. were injected, with negative results. In the experiments reported in this paper 100 young guinea-pigs were used with positive results in many. The average weights of those giving positive results was 200 gm.; of those with negative results 300 gm. The average weight of the rabbits as given in Bull's protocols was 1,580 gm. The average weight of our rabbits showing positive results was 600 gm., while in those showing negative results it was 1,200 gm. Bull injected massive doses of streptococci from tonsils grown aerobically at 37 C. (chiefly on ascites-dextrose agar slants). We injected varying-sized doses from tonsils and brain and cord grown at from 33-35 C. in tall tubes of ascites-dextrose broth affording a gradient of oxygen-tension.

Only 10% of Bull's animals received the primary culture, 70% the second, and 20% the third. In contrast, 70% of the animals reported in this paper received the primary culture. 25% the second, and 5% the third or higher culture generation.

With such differences in methods one is not surprised at the differences in results. However, disregarding the fact that Bull failed to produce symptoms and lesions characteristic of poliomyelitis, localization of streptococci in the central nervous system occurred commonly even in his experiments. Thus, in rabbits, localization occurred more than half as often in the nervous system as in all other organs combined. The blood in his animals, as in ours, was often sterile when brain and meninges showed large numbers of streptococci. That the streptococci from the tonsils of persons who had no obvious symptoms of poliomyelitis, but who resided in the midst of a widespread epidemic, should show streptococci of similar virulence and localizing power is in harmony with results of epidemiologic studies which indicate more and more the widespread prevalence in unrecognized form of the infective agent of this disease.

The symptoms in many of our animals, as shown in the protocols, were quite like those observed in poliomyelitis in man. They were chiefly meningitic in the beginning and later paralytic. Twitchings and spasms of muscles, hyperesthesia and irritability often preceded the onset of paralysis. Death from respiratory failure was common, especially if paralysis began in the anterior extremities. In guinea-pigs improvement in muscle function, with or without contractures, occurred more commonly than in rabbits. In the latter a well established paralysis usually progressed until death. Lesions in other organs were comparatively uncommon (Table 1). The lesions resembled in type those present in poliomyelitis in man and monkey, although in distribution they were not wholly identical. Results comparable to these have not been obtained by any one following inoculations of streptococci from a wide range of sources other than from poliomyelitis or other diseases of the nervous system. That the symptoms and lesions in rabbits must in every way be identical to those in poliomyelitis in man and monkey to be of significance, is open to question. Marks succeeded in passing virus which was adapted to the monkey through a series of six rabbits, producing atypical symptoms and lesions. He was then again able to produce typical symptoms and lesions in the monkey, thus proving that the virus was not modified and that the atypical symptoms and lesions in the rabbit must be

regarded as equivalent to poliomyelitis. The disease induced in rabbits by Marks, and by Rosenau and Havens following inoculation of virus, in guinea-pigs and rabbits by us, and in rabbits by Mathers, and Nuzum and Herzog following inoculation of the pleomorphic streptococcus which is much alike, may therefore be considered as poliomyelitis in these species of animals.

CONCLUSIONS

The streptococcus found so constantly in poliomyelitis tends to localize electively in the central nervous system in young guinea-pigs, rabbits, puppies and kittens, and less often in adult animals of these species and in adult monkeys. The symptoms and lesions produced resemble those found in poliomyelitis in man.

Cultivation on artificial media, especially under aerobic conditions, usually destroys promptly the elective localizing power, as does successive animal passage. The results obtained indicate strongly that the streptococcus under consideration is not merely a secondary invader, but that it has etiologic significance in poliomyelitis.

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EXPLANATION OF PLATES 9-13

Fig. 1.—Guinea-pig 262 and normal control. Flaccid paralysis of hind legs and muscles of the back 3 days after the intravenous injection of the primary culture from the tonsil (see protocol).

Fig. 2.—Guinea-pig 264. Flaccid paralysis of the left foreleg 24 hours after the intravenous injection of pleomorphic streptococcus from a single colony on blood agar from tonsil pus (see protocol).

Fig. 3.—Guinea-pig 337. Paralysis of the left hind leg with contractures and wasting of the hind quarters 14 days after intravenous injection of the pleomorphic streptococcus from a single colony from the cord of Case 707, after one animal passage (see protocol).

Fig. 4.—Guinea-pig 384. Flaccid paralysis of the hind legs and right foreleg with marked weakness of the muscles of the back, 3 days after intravenous injection of the streptococcus from the tonsil after one animal passage (see protocol).

Fig. 5.—Guinea-pig 396. Flaccid paralysis of the right hind leg 48 hours after intravenous injection of streptococcus from the brain of Case 714, after two animal passages (see protocol and text Figure 1).

Fig. 6.—Marked perivascular round-cell infiltration of blood vessels in the brain of Guinea-pig 374. Hematoxylin and eosin. × 200.

Fig. 7.—Streptococci in the area of perivascular infiltration shown in Figure 6. Gram-Weigert. × 1000.

Fig. 8.—Streptococci adjacent to the area of hemorrhage in the cord of Guinea-pig 352. Gram-Weigert. × 1000.

Fig. 9.—Diplococci adjacent to the dilated capillary in the cord of the guinea-pig injected intravenously 5 days previously with a culture from the spinal fluid of Guinea-pig 324. Gram-Weigert. × 1000.

Fig. 10.—Rabbit 952. Flaccid paralysis of Aug. 6, 1916, 4 days after intravenous injection of the primary culture from the tonsil of Case 693.

Fig. 11.—Rabbit 999. Flaccid paralysis of the extremities, 4 days after intravenous injection (see protocol).

Fig. 12.—Rabbit 1017. Flaccid paralysis of fore extremities, 2 days after intravenous injection (see protocol).

Fig. 13.—Hemorrhage and leukocytic infiltration of the pia and round-cell infiltration in the anterior horn of the spinal cord in rabbit shown in Figure 12. Methylene blue and eosin. × 50.

Fig. 14.—Rabbit 939. Round-cell infiltration of the cerebellum 16 days after intravenous injection of the streptococcus from the tonsil of Case 686. Methylene blue and eosin. × 100.

Fig. 15.—Diffuse and perivascular round-cell infiltration in the cerebellum of Rabbit 1001 (see protocol). Methylene blue and eosin. × 50.

Fig. 16.—Same as Figure 15. Higher magnification of blood vessel showing round-cell infiltration. Methylene blue and eosin. × 1000.

Fig. 17.—Round-cell infiltration in the cerebellum of Rabbit 985 (see protocol). Hematoxylin and eosin. × 250.

Fig. 18.—Chain of streptococci in the anterior horn of the spinal cord of Rabbit 980 (see protocol). Gram-Weigert. × 1000.

Fig. 19.—Circumscribed and perivascular infiltration by round cells in the pons of Rabbit 1018 (see protocol). Methylene blue and eosin. × 200.

Fig. 20.—Diplococcus adjacent to the area of hemorrhage in the brain of a rabbit 3 days after intracerebral inoculation with Berkefeld filtrate of the brain of Monkey 10 (see text Figure 1). Gram-Weigert. × 1000.

Fig 1

Fig 2

Fig 3

Fig 4

Fig 5

PLATE 10

Fig 6

Fig 7

Fig 8

Fig 9

PLATE 11

Fig 10

Fig 11

Fig 12
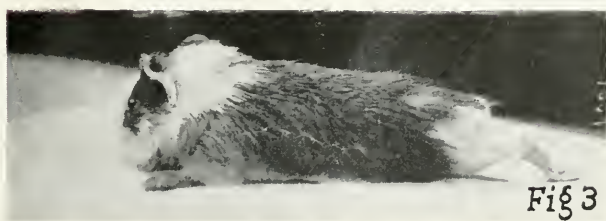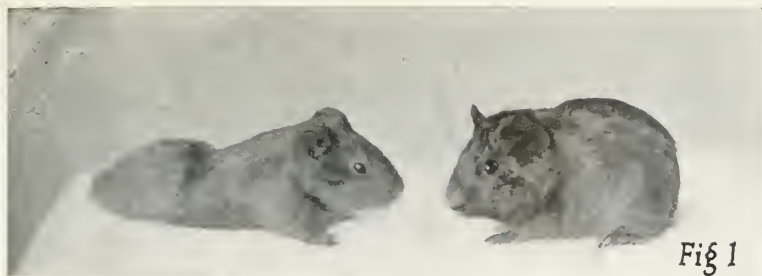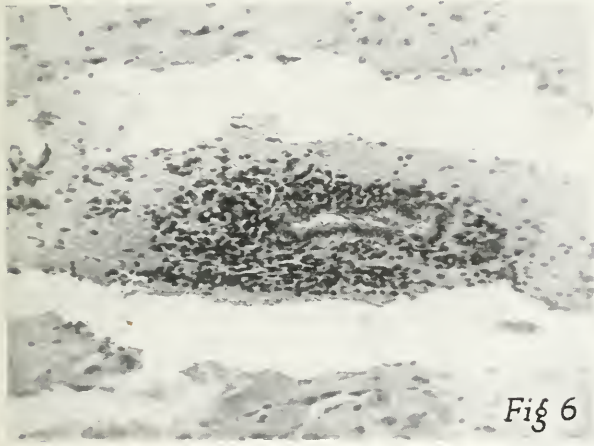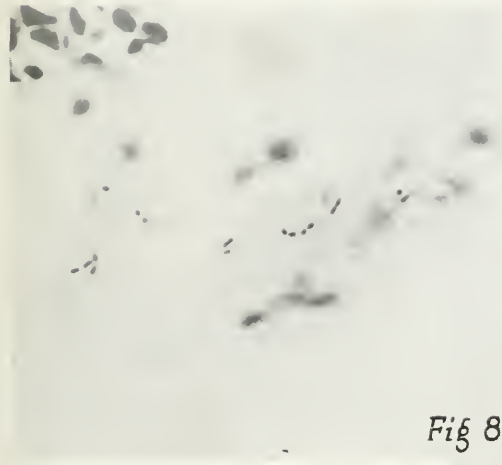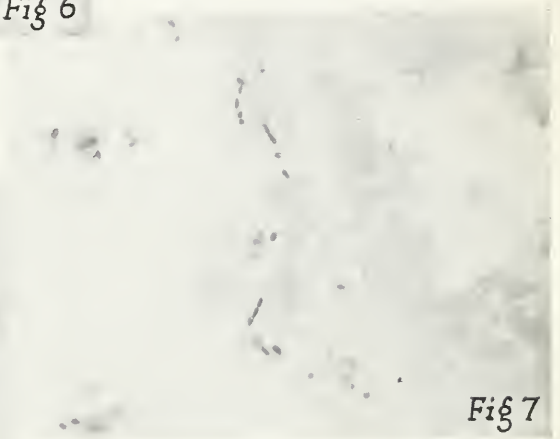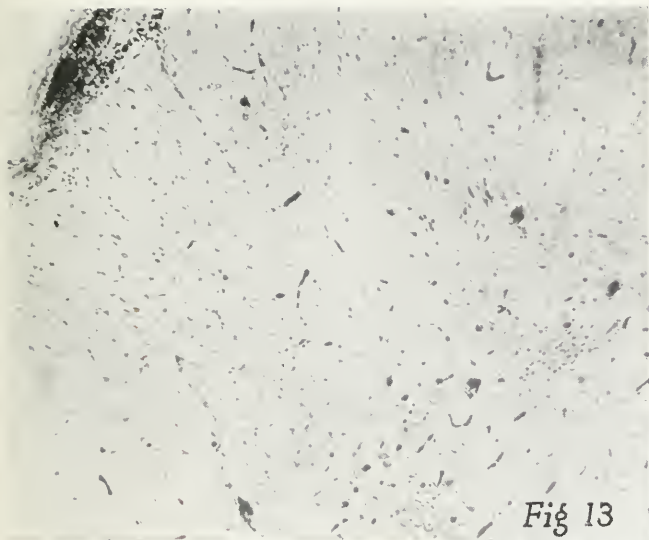
PLATE 12

Fig 13
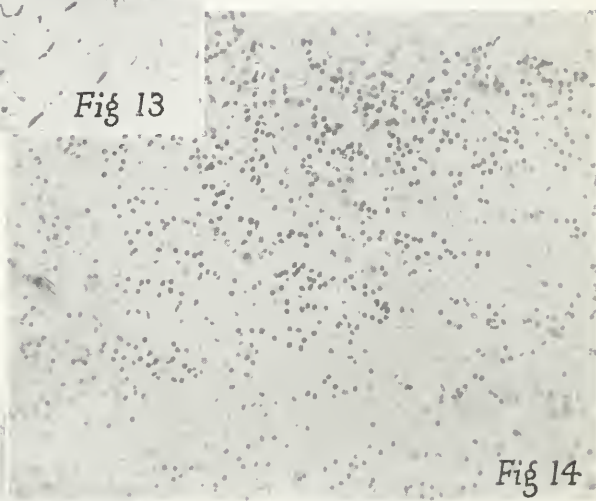
Fig 14

Fig 15

Fig 16

PLATE 13
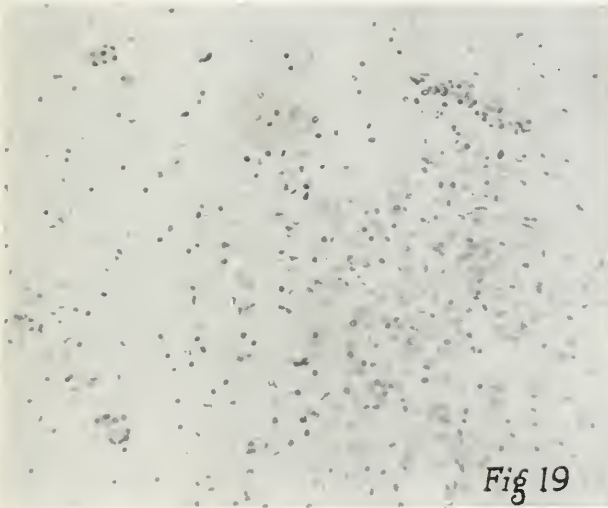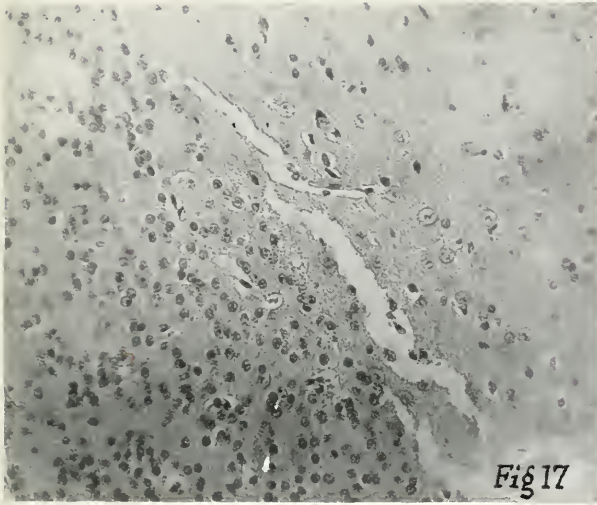
Fig 17

Fig 18

Fig 19

Fig 20

AGGLUTINATION OF THE PLEOMORPHIC STREPTO-COCCUS ISOLATED FROM EPIDEMIC POLIO-MYELITIS BY IMMUNE SERUM

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Recent investigations on the bacteriology of poliomyelitis have shown quite constantly in the atria of infection and in the infected tissues of epidemic poliomyelitis a pleomorphic streptococcus or micrococcus which, soon after isolation, has tended to localize electively in the central nervous system of animals and to produce paralysis. These properties are soon lost on cultivation. The importance of immunologic studies was recognized early and Sept. 11, 1916, immunization experiments were begun by the injection of a monkey with the pleomorphic streptococcus in order to protect it against virus should it recover from the effects of the injection.[1] Since then, horses and monkeys have been immunized and their serum tested for immune bodies (chiefly agglutinins), for neutralizing and protecting power over virus, and for curative effects on experimental poliomyelitis in monkeys. The agglutinating power of the serum of patients and monkeys that have recovered from poliomyelitis, together with numerous normal controls, and its curative effect on poliomyelitis in man has been studied. Summaries of these studies have already been published.[2, 3] In this paper we wish to record in greater detail the experiments and results obtained.

Technic

In the immunization of horses increasing doses of the pleomorphic streptococcus were injected intravenously on 3 consecutive days, with an interval of a week between each series from November 2 to May 1. The bacteria used for the injections were grown in dextrose broth or ascites dextrose broth for 24 hours, then centrifugalized out and suspended in salt solution.

Horse 1 was injected from Nov. 2, 1916, to May 1, 1917. At first strains from human poliomyelitis (heated to 60 C.) were injected, and for a short time both human and monkey strains were used. For many weeks, however, live cultures of strains from experimental poliomyelitis in monkeys, were injected exclusively. Test bleedings were made November 2, 4 and 22, December 22, January 8 and 30, March 3, April 3, and May 14 and 16. The serum obtained

December 22, 7 weeks after immunization was begun, agglutinated the pleomorphic streptococcus in dilutions of 1 : 6150 and showed neutralizing and protecting power over virus.

Horse 2 was injected from November 22 to December 13 with live cultures from human and monkey poliomyelitis. During the injection, December 13, the animal died from acute anaphylactic shock. It was bled before the injection, and soon after death.

Horse 3 was injected from January 30 to May 12 with strains from human poliomyelitis. Test bleedings were made January 30, March 3, April 3, and May 14.

There was difficulty in preparing satisfactory antigens for agglutination purposes. The pleomorphic streptococcus, as pointed out in the preliminary report,[3] tends to grow in clumps or to clump spontaneously in salt solution suspensions. In attempting to overcome this difficulty the character of growths in various liquid mediums was studied under various conditions, and it was found that after incubating from 33-35 C., tall columns of dextrose broth or ascites dextrose broth (0.2% dextrose, 0.6% acid to phenolphthalein, 10% ascites fluid) growth was usually diffuse and marked at the end of from 16-24 hours.

By now neutralizing the broth to phenolphthalein with sodium hydroxid and placing it in the ice chest, or by centrifugalizing out the bacteria and making dense suspensions in normal salt solution the difficulty was largely overcome. The common 6 ounce nursing bottle filled to a depth of 9.5 cm. containing 150 c.c. of broth was found most satisfactory. The tendency to clump-formation was, for unknown reasons, less in these containers than in test tubes containing the same medium, even in columns equally tall. Growth almost invariably began in the deeper layers and would reach the top first, after the growth pressure was at its maximum. Centrifugalization or neutralization was usually done when the growth had extended to within 1-0.5 cm. of the top. The suspensions used as stock solutions were made so that 1 c.c. of the suspension contained the bacteria from 15 c.c. of the broth culture. The antigens in the experiments, unless otherwise mentioned, consisted of the stock suspensions diluted with salt solution immediately before using to the density of the broth culture. The ascites dextrose and dextrose-broth cultures, while fresh, were almost as satisfactory as salt solution suspensions, but spontaneous clumping usually occurred even after neutralization in from a few days to a week or occasionally longer. The bacteria remained in a suitable condition for agglutination tests for a long time in the salt solution suspensions when kept in the ice chest. This was an important point for the specific agglutinating property tends to be lost on artificial cultivation especially aerobic cultivation. Equal parts (usually 0.2 c.c. each) of bacterial suspension or antigen and serum or dilution of serum were thoroughly mixed. Two types of dilutions of serum were used: Progressive 1 : 5, and progressive 1 : 10 dilutions, beginning with equal parts of serum and antigen. The mixtures were incubated for 1½ hours. In the earlier part of the work a reading was made and the tubes placed in the ice chest over night after which a second reading was made. The latter was found to be more reliable and was ultimately adopted. The readings were made against a black background through intense transmitted light from a 100 watt nitrogen bulb so shaded as to protect the eyes. The degree of agglutination is indicated in the tables by 1 or more plus (+) signs, no agglutination by 0. One plus sign

Fig. 1.—Photomicrograph of a hanging drop of mixture of a suspension of the pleomorphic streptococcus (M 49.4) and normal monkey serum (M 121) diluted 1:100. Note the even distribution of the bacteria. This mixture had been incubated 1½ hours and then kept in the ice chest over night ($\times$ 200).
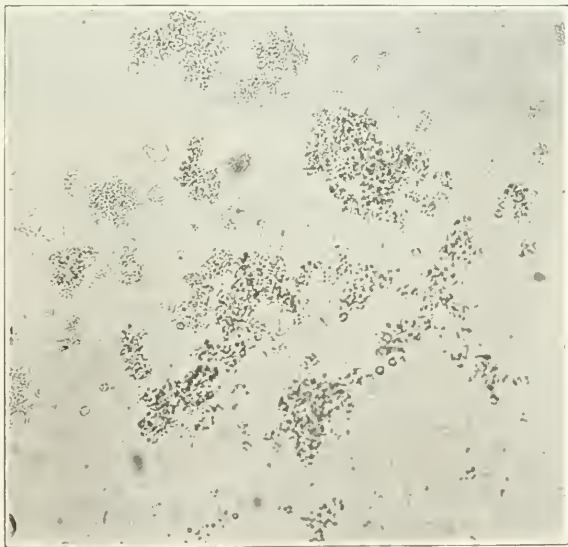
Fig. 2.—Same as Fig. 1, but with the serum of monkey (M 105) paralyzed with virus. Note the marked agglutination of the bacteria. The reading of result in case of Fig. 1 in the macroscopic test was 0, in Fig. 2 ++ ($\times$ 200).

indicates an undoubted clumping, 2 plus signs clumping with some clearing of
the mixtures, 3 plus signs marked clumping with complete clearing of the mix-
tures, and 4 plus signs the same as 3 except that the clumps were larger. The
value of the signs is well illustrated in Figure 3. At first agglutination was
studied in hanging drop, to make sure that what appeared as macroscopic agglu-
tination meant a real clumping of the streptococci (Figs. 1 and 2). In the low
dilutions of the immune serum and antigen there was often an increased cloud-
ing of the mixture with or without precipitation. This is indicated in the tables
by a "C" affixed to the sign of agglutination. Parallel tests were made with
the serum of normal animals corresponding to the species from which the
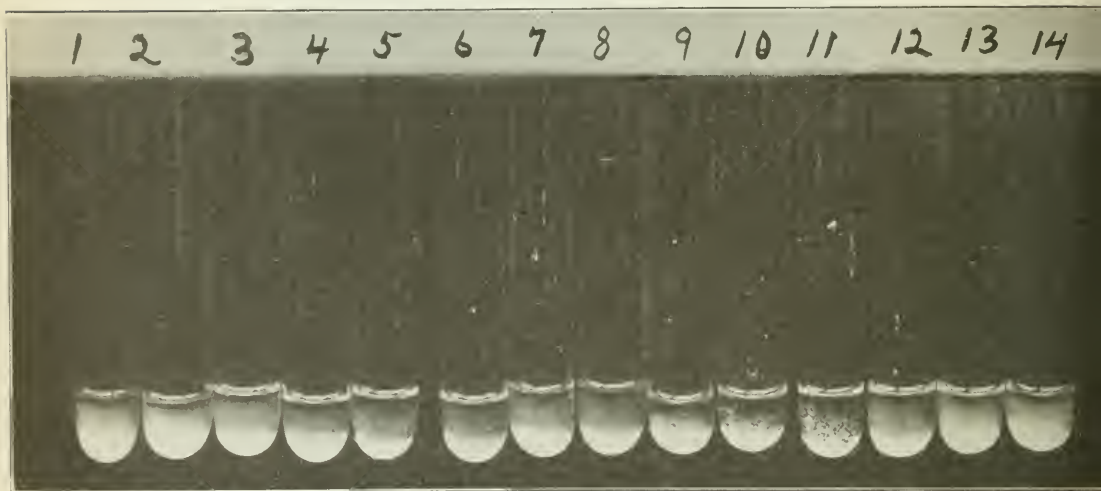
Fig. 3.—Agglutination of human (721.4².2) strain by the serum of Horse 1 (Tubes 1-7)
and of monkey strain (M 49.4) with the serum of Horse 3 (Tubes 8-14). The first tube in
each series contained equal parts of antigen, normal salt solution, suspensions of the bacteria,
and serum; the others contained equal parts of antigen and progressive 1: 10 dilutions
of serum in normal salt solution. Note the increasing agglutinating power of these serums
with increasing dilutions up to 1: 10,000 and its rapid disappearance in dilutions above this
point, there being slight agglutination in 1: 100,000 dilutions but none in 1: 1,000,000
dilutions.

immune serum was obtained. Controls in normal salt solution and control
strains of streptococci were included and in addition the agglutinating power of
antipneumococcus and antistreptococcus serum over the pleomorphic strepto-
coccus was tested. In the text and tables, for the sake of simplicity, the expo-
nent affixed to a given number of animal or strain indicates animal passage
whereas the figure after the period indicates the culture generation; thus
714².3 indicates that this strain has been passed through one animal and that
it is in the third culture generation. For the same reason the expressions
"human" and "monkey" poliomyelitis are used. "Virus immune" monkeys are
those injected with emulsions or filtrates of poliomyelitic brain and cord and
which developed typical poliomyelitis; "culture immunes" are those which were
injected with cultures of the pleomorphic organism.

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS
BY THE SERUM FROM HORSES

In Table 1 is given the agglutinating titer of the serum from Horse 1 (immunized chiefly with monkey strains), against a strain from human poliomyelitis. The strain used in this experiment was isolated a short time previously from the dried brain of a patient (Case 714). The agglutinating power of this serum before injection and 2 days after the first injection corresponds quite closely to what has been observed in the serum of normal horses. The increase in agglutinating power of the serum in the successive bleedings is well shown. Similar results were obtained with other strains and with the serum from Horse 2 and Horse 3. The agglutinating titer of the

TABLE 1

Agglutinating Titer of the Serum of Various Bleedings from Horse 1 over the Pleomorphic Streptococcus (714) from the Brain in Human Poliomyelitis

Dilutions of Serum	Serum from Horse 1						
	Nov. 2	Nov. 4	Nov. 22	Dec. 22	Jan. 8	Jan. 30	March 3
1 : 1	+++	+++	+++	+	+c	+c	++c
1 : 10	++	++	++++	++	++	++	+++c
1 : 50	0	0	++	++++	++++	++++	++++
1 : 250	0	0	++	+++	++++	++++	+++
1 : 1250	0	0	0	+	+	+++	+
1 : 6150	0	0	0	+	0	++	+
1 : 30,250	0	0	0	0	0	0	+

TABLE 2

Agglutination of the Pleomorphic Streptococcus from Human Poliomyelitis (714) Under Various Conditions

Serum from	Dilutions of Serum	Dextrose Broth Culture		Suspension of NaCl Solution					
				Of the Density of the Broth Culture					15 Times the Density of the Broth Culture
		Acidity of 48 Hour Culture	Neutralized to Phenolphthalein	Untreated	Heated to 60 C. 30 Min.	Heated to 100 C. 3 Min.	+ .5% Formalin	+ .5% Phenol.	
Normal Horse	1 : 1	+++	++	+++	+++	+++	+	+++	++
	1 : 10	++	+	++	++	+++	+	++	+
	1 : 50	0	0	+	0	++	+	0	0
	1 : 250	0	0	0	0	0	0	0	0
	1 : 1250	0	0	0	0	0	0	0	0
Horse 1 (Jan.30)	1 : 1	+	0	+	++	++	+	+	+++
	1 : 10	+++	++	+++	+++	+++	++++	++	++++
	1 : 50	++++	++++	++++	++++	++++	+++	+++	++++
	1 : 250	+++	++++	++++	+++	+++	+++	++	++++
	1 : 1250	+++	+++	+++	+	+++	?	+	+

serums on April 3 was no higher than on March 3. It is of interest to note that at about this time the horse was losing some weight and had developed a multiple arthritis. It appeared as if the injections were too large, for after an interval of 3 weeks of no injections and then the injections of smaller doses, the agglutinating titer had again increased by May 14.

In Table 2 are given the results of an experiment which shows that a proper antigen may be treated in various ways without destroying the agglutinability of the streptococci. The agglutinations in the broth culture of the original acidity and after neutralization to phenol-phthalein ran quite parallel, differing only slightly from those in the salt solution suspensions of the density of broth culture when the latter were untreated, when they were heated to 60 C. for 30 minutes, when they were heated to 100 C. for 3 minutes and when 0.5% formalin or 0.5% phenol was added. The suspension 15 times the density of the broth culture also yielded comparable results.

In this connection, one point should be emphasized. In Table 1 it may be seen that as the agglutinating titer of the serum increased there was decrease in agglutination in the low dilutions. Instead of agglutination there was often a marked increased cloudiness or precipitation. This observation was made with numerous strains and is being studied in greater detail. Given a strain which was sharply agglutinated in the 1 : 1 and 1 : 10 dilution of normal horse serum, there was often no agglutination or less agglutination in these dilutions of the highly immune serum, but marked agglutination in the higher dilutions (Table 2 and Figs. 3 and 4). If there was no agglutination of certain strains by normal horse serum, the maximum agglutination by the highly immune serum was shifted toward the lower dilutions. Moreover, if the amount of antigen was greatly increased, agglutination tended to be less marked in the low dilutions of normal serum, while in the immune serum in the low dilutions it became more marked (Table 2). It appears, therefore, that no agglutination may be the result of either too little or too much agglutinin, and that antigen and antibody to be effective must be present in definite proportions.

The antigen used in Table 2 was freshly prepared. As previously indicated it was the custom to prepare large amounts of antigen when the condition of the strain was thought to be right, and to preserve

the unused portion in the ice chest. After determining that the agglu-
tinating power of the serum from Horse 1 and Horse 3 on May 14 was
comparable to that of previous bleedings, an experiment was performed
to test the keeping qualities of various antigens. Twenty-one antigens
—17 from human sources, 2 from monkey sources, and 2 controls—
were titrated against normal horse serum and the serum of Horse 1
and Horse 3. The interval between the primary agglutination test and
this experiment ranged from 50-126 days. To 17 of the antigens, 0.5%
phenol was added at the time of preparation. The rest were suspen-
sions in salt solution and still contained living bacteria. The agglutina-
tion by the normal horse serum was about the same in the fresh and
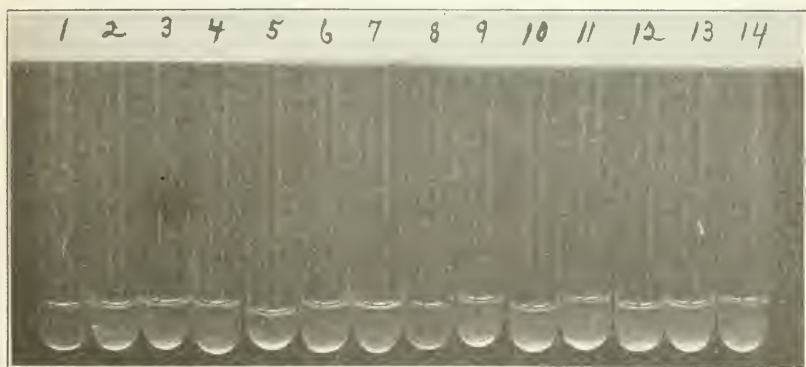
Fig. 4.—Same as Fig. 3 with normal horse serum. Note the complete absence of
agglutination of the human strain and slight agglutination in the 1:1 and 1:10 dilutions of
the monkey strain.

in the preserved antigen. The agglutination by the serum of Horse 1
was not materially changed in 6, was moderately reduced in 7, and
markedly less or entirely absent in 6 antigens. The agglutination by
the serum from Horse 3 was not materially less than in the original
test in 9 instances, moderately less in 5, and markedly less or entirely
absent in 5. The loss or maintenance of agglutinability toward the 2
immune serums usually ran parallel. The control antigens showed
little or no agglutination. In one instance in which strains from both
the tonsil and cord were used the agglutination was markedly reduced
in both. Reduction in agglutinability occurred irrespective of whether
the bacteria were alive or dead from addition of phenol.

EXPERIMENTS ON THE AGGLUTINABILITY OF THE STREPTOCOCCUS ISOLATED FROM BRAIN AND CORD OF PARALYZED ANIMALS

The pleomorphic streptococcus was often isolated in large numbers from the brain and cord of animals showing paralytic symptoms when cultures of the blood and other tissues showed few or no organisms. The morphologic and other characteristics of these strains, while quite distinctive when first isolated, were too temporary and sometimes not sufficiently striking to differentiate them unmistakably from strepto-

TABLE 3

Agglutination of the Pleomorphic Streptococcus (714) Before and After Animal Passage by the Serum of Horse 1

Serum from	Dilutions of Serum	Before Animal Passage				After Animal Passage—			
		714.3				$714^3.7$	$714^3.3$		
		Recent Culture, Dried Brain	Old Anaerobic Culture, Fresh Brain	714.3 Cerebral Fluid	714.6 Cerebral Fluid	R985	R998	R999	R1000
Normal Horse	1 : 1	++	+	+	+	+	++	+	+++
	1 : 10	+	0	+	+	++	0	0	+
	1 : 50	0	0	0	0	0	0	0	0
	1 : 250	0	0	0	0	0	0	0	0
	1 : 1250	0	0	0	0	0	0	0	0
	1 : 6250	0	0	0	0	0	—	—	—
	1 : 31,250	—	—	0	0	0	—	—	—
Horse 1	1 : 1	+c	+c	+++	0c	++	0c	0c	0c
	1 : 10	++	++c	+++	+c	++	0	+	+
	1 : 50	++++	++c	+++	+	++	+++	++	++++
	1 : 250	+++	+++	+++	++	+	++	++	++++
	1 : 1250	+	+	++	++	0	+	+	+
	1 : 6250	+	0	+	0	0	—	—	—
	1 : 31,250	—	—	+	0	0	—	—	—

cocci which produce green colonies on blood agar. The possibility of the occasional presence of other streptococci in small numbers in the brains of these animals was recognized and the need, therefore, for immunologic differentiation was apparent in the early part of the work. At the time of necropsies on paralyzed animals the aspirated brain substance and often also a small amount of ventricular or spinal fluid was filed away in sealed pipets in the refrigerator. It was thought, too, that peculiar properties of these strains might best be maintained when they were kept in this condition. Many of the strains from human poliomyelitis which were used for the immunization of Horse 3 and for the preparation of antigens used in these experiments had been kept in this manner until shortly before performing the experiments. The results of the agglutination experiments with these strains before and after animal passage are now to be given in some detail.

Table 3 shows the agglutinating property of one strain (714) from typical poliomyelitis in a child before and after animal passage, about 5 months after isolation, together with controls. The strain used in the first column had been isolated a short time previously from a piece of the dried brain. The one in the second column was isolated from the fresh brain at the time of necropsy and was preserved in a deep culture of ascites plain tissue broth covered with oil. The strain in the third and fourth columns was isolated from the cerebral fluid at the

TABLE 3—*Continued*

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS (714) BEFORE AND AFTER ANIMAL PASSAGE BY THE SERUM OF HORSE 1

—After Animal Passage							Controls		
714^a.3		714^b.3							
			M12		748^2				
R1017	R1023	M10 Brain	Brain	Axillary Lymph Gland	Tonsil R1037	756 Teeth	756^2 R1039	622	257
+++	++	+++	+++	+++	+	++	0	+	0
+	+	++	0	++	0	+	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
—	0	0	0	0	0	0	—	—	—
—	0	0	0	0	0	0	—	—	—
0c	+c	0c	+c	+c	0	0	0c	0	0
0c	++	+c	+c	+++	0	0	+	0	0
+++	+++	++++	++++	+++	0	0	0	0	0
++++	+++	+++	+++	++	0	0	0	0	0
+	++	++	++	0	0	0	0	0	0
—	+	+	+	0	0	0	—	—	—
—	0	+	0	0	0	0	—	—	—

time of necropsy and was preserved in an ascites plain tissue agar stab. The former was in the third subculture, the latter in the sixth. All of these were agglutinated markedly and in high dilutions by the serum from Horse 1, immunized with monkey strains; the one in the sixth culture less markedly than the one in the third. The identification of the strains isolated from animals is so important that the results of the animal experiments are given somewhat in detail.

An emulsion of the fresh brain was injected August 24 into the brain of a young rabbit (978). It developed flaccid paralysis of the left fore leg on the 4th day, and died of respiratory failure. Cultures from the blood of this rabbit were sterile, while those from the brain showed the pleomorphic streptococcus in pure culture. Cultures from the brain in the first and second generation were injected intravenously August 30 into each of 4 young rabbits (985, 998, 999, 1000), the doses ranging from 1 c.c. of culture to the growth from 30 c.c. of culture.

Rabbit 985 developed an ascending flaccid paralysis and died of respiratory failure. Cultures on blood agar plates of the brain substance at the time of necropsy showed enormous numbers of small dry green colonies of the streptococcus. This strain was grown for 6 generations on blood agar and the antigen prepared from the next, or seventh, culture in ascites dextrose broth. It was still in an agglutinable condition as shown in the table.

The strains from Rabbits 998, 999 and 1000, from which the antigens were prepared, had been isolated a short time previously from the brain material which had been preserved in the ice chest for about 6 months. The antigens were prepared from the second dextrose broth culture after one plating on blood agar. Rabbit 998 died 48 hours after injection. Cultures from the blood were sterile, those from the fresh brain showed a large number of green colonies of streptococci, and those from the preserved material showed a few similar colonies. Rabbit 999 developed ataxia, coarse tremor of the head and forelegs on the second day, complete flaccid paralysis of the hind legs and left foreleg on the fourth day, and was found dead on the fifth day. Cultures from the blood were sterile, those from the fresh brain showed 5 typical green producing colonies on blood agar, and the preserved material showed no growth on aerobic blood agar plates and a few green colonies on an anaerobic blood agar slant. Rabbit 1000 was found dead the day after a second injection. Cultures on blood agar plates of the blood were sterile, those of the fresh brain showed 5 green colonies of streptococci, while those from the preserved brain material showed 15 similar colonies. The strains isolated from the fresh and preserved brain of these 3 rabbits appeared identical on blood agar, all producing dry, green colonies of streptococci, which were agglutinated markedly and in high dilutions by the serum from Horse 1.

Rabbit 1017 was injected intravenously September 5 with the growth from 15 c.c. of broth culture from the brain of Rabbit 999. The animal became unsteady and weak on the second day; there was constant tremor of the head and it was just able to stand. The next day it was worse; power in the front legs and left posterior neck muscles was gone and it pushed itself along with the hind legs. It died on the third day. Blood-agar plate cultures from the fresh brain showed innumerable fine green colonies of streptococci, those from the kidney many colonies and those from the blood a few similar colonies. No streptococci were obtained from the liver and joint fluid. The preserved brain material showed countless numbers of green colonies of streptococci. This strain and the strains from the 3 rabbits in the previous animal passage were agglutinated similarly.

Rabbit 1023 was injected intracerebrally September 8 with 0.5 c.c. of a Berkefeld filtrate of the emulsion of the fresh brain of Rabbit 1017; Monkey 10 was injected intracerebrally with 1.5 c.c. of the emulsion; and Monkey 12 was injected intravenously with the growth from 30 c.c. and 60 c.c. of ascites dextrose and plain broth culture from the brain of the same rabbit. Rabbit 1023 remained well for 48 hours. It then developed meningeal and cerebellar symptoms with marked weakness of the adductors of the right hind leg, and died on the third day. Blood-agar plate cultures of the fresh brain and lumbar cord showed countless fine dry green colonies of streptococci in pure culture, while those from the filtrate of the brain and cord emulsion yielded the same organism also in pure culture. The culture from the preserved brain material showed many identical colonies. Cultures from the blood, liver and kidney showed no streptococci.

Monkey 10 developed flaccid paralysis beginning the day after the injection, which extended rapidly until death occurred from respiratory failure. Blood-

agar plate cultures of the fresh brain and cord and of the edematous fluid surrounding the cord showed countless fine dry green colonies of streptococci in pure culture. The cultures from the blood, kidney, spleen and lymph gland were negative. Those from the preserved brain material showed a moderate number of green colonies.

Monkey 12, as previously reported, developed flaccid paralysis beginning in the left arm the day following the injection. This extended and the animal became prostrate on the sevnth day, when it was chloroformed. Cultures from the brain and axillary lymph gland yielded the characteristic organism. Both were preserved in deep cultures until shortly before the agglutination tests were made. This strain in the fourth animal passage from the brain of Rabbit 1023 from the brain of Monkeys 10 and 12 and from the axillary lymph gland of the latter produced green colonies on blood agar at the time of the tests. All were agglutinated markedly and in high dilution as shown in Table 3. The control strain 748 was isolated from the blood of Rabbit 1037, injected with the emulsion of the tonsil from a patient with poliomyelitis 6 weeks after the attack. The rabbit showed no paralysis. The control strain 756, a green producing streptococcus, was isolated from the pyorrheal pockets about the teeth of a monkey with symptoms suggesting poliomyelitis, but sections of the cord showed no lesions. This strain was injected intravenously into Rabbit 1039. No paralytic symptoms were noted. The strain not passed through animals was cultivated on blood agar while the strain after animal passage was preserved in the brain material of Rabbit 1039 and isolated a short time previous to the experiment. The control strain 622 was a pneumococcus and the control strain 257 a hemolytic streptococcus. None of the control strains were agglutinated. Results similar to those given in Table 3 were obtained in numerous instances with other antigens prepared from subsequent cultures of the preserved brain of these animals, and, as will be seen, the condition of many of these strains was such that agglutination took place with the serum of patients and monkeys which had recovered from attacks of poliomyelitis.

According to the agglutination tests the specific organism was isolated in this case of human poliomyelitis, (1) from the fresh brain by direct culture and by the injection of an emulsion into the brain of a rabbit, (2) from the dried brain, months later, and (3) from the cerebral fluid at the time of necropsy. It was possible to recover the organism in the second animal passage from the brain of each of 4 rabbits; in the third animal passage, from the brain of 1 rabbit injected with the filtrate of the brain emulsion of one of these; in the fourth animal passage, from the brain of 1 rabbit and 1 monkey, and from the brain and the axillary lymph gland of another monkey. The organism in all of these 7 animals tended to localize electively in the central nervous system.

Table 4 shows the agglutinating power of immune serum from Horse 1 and Horse 3 over another strain (722) from human poliomyelitis before and after animal passage. All of the strains which were agglutinated produced fine dry green colonies on blood-agar plates

TABLE 4

Agglutination of the Pleomorphic Streptococcus (722) Before and After Animal Passage by the Serum from Horse 1 and Horse 3

Serum from	Dilutions of Serum	Before Animal Passage		After Animal Passage					
		722.7 Brain	722.8 Brain	722².2 (Brain) R1003 Green-producing Streptococcus	722².3 (Brain) R1003 Indifferent Streptococcus	722².3 (Brain) R1015 Hemolyzing Streptococcus	722².4 (Tonsil) P414	722².4 (Cord) P420 Hemolytic Streptococcus	722³.3 (Brain) R1010
Normal Horse	1 : 1	++	++	+++	0	+	0	+	++
	1 : 10	+	+	++	0	0	0	0	++
	1 : 100	+	0	+	0	0	0	0	0
	1 : 1000	0	0	0	0	0	0	0	0
	1 : 10,000	0	0	0	0	0	0	0	0
	1 : 100,000	0	0	—	—	0	0	0	0
	1 : 1,000,000	0	0	—	—	0	0	0	0
Horse 1	1 : 1	+c	++	+	0	+	+++	+c	+++
	1 : 10	+	++	+	0	0	++	0	++++
	1 : 100	++	+++	++	0	0	+	0	++++
	1 : 1000	+	+++	++	0	0	0	0	+
	1 : 10,000	0	+	+	0	0	0	0	0
	1 : 100,000	0	0	—	—	0	0	0	0
	1 : 1,000,000	0	0	—	—	0	0	0	0
Horse 3	1 : 1	++	+++				++++		+++
	1 : 10	+++	+++				++		++++
	1 : 100	++	+++				+		++++
	1 : 1000	0	+				0		++
	1 : 10,000	0	0				0		0
	1 : 100,000	0	0				0		0
	1 : 1,000,000	0	0				0		0

at the time the tests were made, just as they did when first isolated. The strain in the seventh and eighth culture which had not been passed through animals had been grown in ascites tissue agar stabs and on blood-agar slants from Sept. 2, 1916, until March 21, 1917. Results similar to those shown in the table were obtained with 4 other antigens from this strain. The strain after 1 or 2 animal passages was preserved in the brain substance placed in sealed pipets in the ice chest until shortly before the tests were made. This material was then plated on blood agar, planted in dextrose or ascites dextrose broth and used for the agglutination tests in the second, third, or fourth culture generation.

Rabbit 1003, 470 gm., was injected intracerebrally Sept. 1, 1916, with 0.25 c.c. of the emulsion of the brain of a patient with typical poliomyelitis (Case 722). The animal died within 24 hours in convulsions, after marked antemortem weakness of the hind extremities. Cultures of the blood were sterile; cultures from the fresh brain yielded a pure growth of fine dry green colonies on blood agar; while those from the preserved material showed 2 types of colonies— a moderate number of fine dry green, and a few indifferent colonies of streptococci. The former type was agglutinated in high dilution by the serum from Horse 1, the latter not at all.

Rabbit 1015, 250 gm., was injected intravenously Sept. 5, 1916, with a scant growth from 30 c.c. of ascites dextrose tissue broth of the streptococcus (in the third generation) from the brain (Case 722). The animal died within 24 hours. There was hemorrhagic edema surrounding the cord and marked softening of the brain and cord. A few small embolic foci were found in the right ventricle and a number of small hemorrhages in the lungs. Cultures of joint fluid, blood, liver, kidney and spleen remained sterile, while the brain and lumbar cord showed countless fine dry green colonies. The preserved brain material showed very many slightly hemolyzing colonies of streptococci distinctly different from those isolated at the time of necropsy. The third culture generation of this strain as shown in Table 4 was not agglutinated by the serum from Horse 1.

Guinea-pig 414 was injected intracerebrally Sept. 1, 1916, with 0.25 c.c. of a salt solution emulsion of the tonsil (Case 722). The animal showed marked weakness, tremor and irritability, and died on the sixth day. Marked extradural hemorrhage in the cervical region of the cord, and a bronchopneumonia were found. There were no lesions of joints or nerves. Cultures from the blood showed 5, from the brain enormous numbers, and from the cord a few fine dry green colonies. The cultures from the lung and liver showed colon bacilli. Blood-agar plate cultures from the preserved brain material remained sterile, but the ascites dextrose broth culture showed the streptococcus and colon bacillus. Plating of this culture showed green colonies of the streptococcus. The agglutination of this strain in the fourth culture was marked in both immune serums although not in dilution over 1 : 100.

Guinea-pig 420 was injected intravenously Sept. 2, 1916, with the growth from 10 c.c. of the primary ascites dextrose broth culture of the cord. Plate cultures of the emulsion injected showed colon bacilli and typical hemolytic streptococci. The animal died in 7 hours. Cultures of the blood and brain showed hemolytic streptococci and colon bacilli; those from the preserved brain material also showed hemolytic streptococci as did the antigen used for the agglutination tests. This strain, although kept under exactly the same conditions as the characteristic streptococcus, was not agglutinated.

Rabbit 1010 was injected intravenously Sept. 3, 1916, with 4 c.c. of ascites dextrose-broth culture from the brain of Rabbit 1003. The animal showed flaccid paralysis of the left front leg, and died in convulsions on the third day. There was found marked edema surrounding the dura of the cord, turbid cerebrospinal fluid and slight clouding of the pia. There were no other lesions. Blood-agar plate cultures of the blood remained sterile, while those of the lumbar cord and brain showed a great many green colonies of streptococci, and those from the preserved brain material showed countless numbers of small dry green colonies of streptococci. The agglutination of this strain was marked, and in dilutions up to 1 : 1000.

Results similar to those given in Table 4 were obtained with the green-producing streptococcus from Rabbit 1003 in 3 other experiments, from Pig 414 in 4, from Rabbit 1010 in 14, and from Rabbit 1015 in 2. Agglutination with antipneumococcus serum, Types I and II, of the strains from Rabbits 1003 and 1010, was no higher than with normal horse serum. The strain from Rabbit 1010 was agglutinated by the serum from a case of sporadic poliomyelitis and by the serum from 11 out of 12 cases of epidemic poliomyelitis, but not by the serum from 5 normal persons. The serum of 7 of 12 paralyzed monkeys agglutinated this strain more markedly or in higher dilution, or both, than the serum of 5 normal monkeys used as controls.

Therefore, according to the agglutination tests, the specific strain in this case was isolated from the tonsil by the injection of an emulsion into the brain of a guinea-pig, and from the brain by direct culture and by the injection of an emulsion into the brain of a rabbit and moreover it was re-isolated in 2 successive animal passages.

Judging from the results of the cultures of the brain of Rabbit 1003, the strain producing indifferent colonies on blood agar which was not agglutinated, should be looked on as having lost the characteristic properties, although the possibility of its being a contaminant must be admitted. The strain from Rabbit 1015 undoubtedly acquired hemolyzing power and accordingly lost its agglutinability. The strain from the cord injected into Pig 420 was clearly a contamination.

According to the agglutination tests, the specific streptococcus was isolated from the tonsil, from the brain, and from the cord (Case 721). The strain from the cord was re-isolated in pure culture from the brain of both a monkey and a guinea-pig that had shown paralysis following intravenous injection 13 and 12 days previously. Similar results were obtained from other cases.

To summarize: According to the agglutination tests, the specific organism was isolated from the fresh or preserved brain material in all of 18 animals (13 rabbits, 2 guinea-pigs and 3 monkeys) injected with strains from 8 cases of poliomyelitis. There was a marked tendency for the organism to localize in the central nervous system. The blood from these animals was sterile in all but 4 instances, and in these it contained only a few of the organisms while cultures from the

TABLE 5

Agglutination of Streptococci from Monkey 85

		Serum from		
Strain	Dilutions of Serum	Normal Horse	Horse 1 (Jan. 30) Immunized with Monkey Strains	Monkey 85 Paralyzed with Virus
Green streptococcus from nasal mucous membrane	1 : 1 1 : 10 1 : 50 1 : 250 1 : 1250	0 0 0 0 0	0 0 0 0 0	0 0 0 0 0
Pleomorphic streptococcus from cyst in brain	1 : 1 1 : 10 1 : 50 1 : 250 1 : 1250	0 0 0 0 0	+++ +++ ++ + 0	+ ++ 0 0 0
Pleomorphic streptococcus from cord	1 : 1 1 : 10 1 : 50 1 : 250 1 : 1250 1 : 6250	+ + 0 0 0 0	++ ++ ++++ +++ ++ +	— — — — — —

brain showed large numbers. Thirteen animals showed symptoms referable to the nervous system and all showed gross or microscopic lesions. Emulsions or cultures from tonsils were injected in 4 instances, emulsions of the brain in 3 and cultures from the brain or cord in the rest.

In Table 5 is given a striking example of the specific agglutinating property of the plemorphic streptococcus isolated from the central nervous system of a monkey paralyzed with virus. The green-producing streptococcus isolated from the nasal mucous membrane was not agglutinated either by the serum of Horse 1 or by the serum of the monkey, whereas the pleomorphic streptococcus from the cyst in the brain which also produced green on blood agar, was markedly agglutinated by the serum of Horse 1 and moderately by the serum from the monkey. The strain from the cord also showed marked agglutination with the serum from Horse 1.

TABLE 6

Total Incidence of the Upper Limit of Agglutination of the Pleomorphic Streptococcus from Human Poliomyelitis with the Serum from Normal Horse, Horse 1, and Horse 3, and the Total Incidence of No Agglutination

Dilutions of Serum	Serum from		
	Normal Horse, Percentage	Horse 1, Percentage	Horse 3, Percentage
1 : 1	27	3	5
1 : 10	54	14	13
1 : 100	8	20	44
1 : 1000	0	40	22
1 : 10,000	0	16	11
1 : 100,000	0	2	4
1 : 1,000,000	0	0.5	1
No agglutination	11	3	0

The upper limit of agglutination was found to be a rough index of the agglutinating power of these serums. In Table 6 is given the total incidence of the upper limit of agglutination of the pleomorphic streptococcus isolated from human poliomyelitis with the serum from Normal Horse, Horse 1, and Horse 3, and the total incidence of no agglutination. The results are given in percentage of incidence; hence are directly comparable to those in other tables and need not be discussed in detail. The need of making many tests with the pleomorphic streptococcus and with many control strains was recognized.

Altogether 211 experiments were made testing the agglutinating power of serum from a normal horse and Horse 1 over 92 different antigens prepared from 19 human poliomyelitis strains; 79 of these

TABLE 7

Total Incidence of the Upper Limit of Agglutination of Strains from Monkey Poliomyelitis with the Serum from Normal Horse, Horse 1, and Horse 3, and the Total Incidence of No Agglutination

Dilutions of Serum	Serum from		
	Normal Horse, Percentage	Horse 1, Percentage	Horse 3, Percentage
1 : 1	24	7	11
1 : 10	39	8	14
1 : 100	19	28	32
1 : 1000	0	22	21
1 : 10,000	0	20	11
1 : 100,000	0	5	4
1 : 1,000,000	0	5	0
No agglutination	18	4	1

TABLE 8

Agglutination of Control Strains of Streptococci and Pneumococci by the Serum of Normal Horse and Horse 1

Serum	Dilutions of Serum	Strain and Source							
		999 Chole-cystitis	140 Chole-cystitis	341 Paro-titis	773 Ulcer of Stomach	93 Appen-dicitis	130 Myo-sitis	602 Myo-sitis	1.70 Pneumo-coccus Type I
Normal Horse	1:1	+	+++	++	++	0	+	+	+
	1:10	0	++	+	+	0	0	0	0
	1:100	0	+	0	+	0	0	0	0
	1:1000	0	0	0	0	0	0	0	0
	1:10,000	0	0	0	0	0	0	0	0
	1:100,000	0	0	0	0	0	0	0	0
	1:1,000,000	0	0	0	0	0	0	0	0
Immune Horse 1	1:1	0c	0c	0c	0c	0c	0c	0c	+
	1:10	0	0c	+	0	0	0	0	+
	1:100	0	+	+	0	0	0	0	0
	1:1000	0	+	0	0	0	0	0	0
	1:10,000	0	0	0	0	0	0	0	0
	1:100,000	0	0	0	0	0	0	0	0
	1:1,000,000	0	0	0	0	0	0	0	0

tests included the serum from Horse 3. Forty of the antigens were prepared from 15 strains isolated from brain and cord before animal passage; 30 from 4 strains after animal passage; 15 from 6 strains from tonsils before animal passage; and 7 from 4 strains from tonsils after animal passage. In 6 instances, antigens from the tonsil and brain or cord of the same case were tested. The upper limit of agglutination of these strains with normal horse serum was 1 : 1 or 1 : 10 in nearly all instances. In only 8% of the tests was it 1 : 100, and in none was it above this point. The upper limit of agglutination with the immune serum from Horse 1 was 1 : 1000 or above in nearly 60% of the tests,

and with the immune serum from Horse 3 it was 1 : 1000 in nearly 40% of the tests. In the instances in which no agglutination occurred in the immune serum or in which it was no higher than in the corresponding normal serum, the antigen used had usually been prepared from strains after long cultivation, or after changes in the organism were evident, so that marked agglutination was not to be expected.

In Table 7 is given the total incidence of the upper limit of agglutination of strains from monkey poliomyelitis with the normal and antipoliomyelitic horse serums. Altogether 74 agglutination tests were made with normal horse serum and the serum from Horse 1, 28 of these including the serum from Horse 3. Thirty-nine antigens were used. These were prepared from strains isolated from the central ner-

TABLE 8—*Continued*

AGGLUTINATION OF CONTROL STRAINS OF STREPTOCOCCI AND PNEUMOCOCCI BY THE
SERUM OF NORMAL HORSE AND HORSE 1

Strain and Source

622 Pneumococcus Type II	x11^2 Hemolytic Streptococcus	292 Endocarditis	276 Herpes Zoster	281^2 Herpes Zoster	848 Tonsil	M74 Colitis	B. from Endocarditis		864 Tooth
							Before Animal Passage	After Four Animal Passages	
+++	+	++	++	+++	+	0	+	+	0
+	0	+	0	+	+	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
+c	+c	++	++	+c	++c	0	0	++c	0c
0c	0	++	0	++	0	0	0	++	0c
0	0	0	0	+++	0	0	0	++	+
0	0	0	0	+	0	0	0	+	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

vous system of 20 monkeys that had been paralyzed with virus and 8 monkeys that had received injections of cultures of the pleomorphic streptococcus. The upper limit of agglutination of these strains corresponds quite closely to that of the strains from human poliomyelitis. In the instances in which no agglutination occurred in the immune serum or in which it was no higher than in the normal serum, the antigen had usually been prepared from strains after long cultivation, or after changes in the organism had become evident.

In Table 8 are given the results of agglutination tests of control strains with the serum from Normal Horse and Horse 1. These are

quite representative of the results obtained with a larger series, and include 2 strains (281[3] and B[4]), isolated from cases of herpes zoster and endocarditis, respectively, which were agglutinated in dilutions as high as 1:1000 by the serum from Horse 1. Both had been cultivated for a long time after animal passage. The strain from endocarditis which had not been passed through animals was not agglutinated.

The need for testing the agglutinability of many strains of streptococci from a wide range of sources with these serums was apparent. Pneumococci of Types I and II according to Cole, hemolytic streptococci and Streptococcus mucosus were included in the control series. Altogether 127 agglutination experiments with 103 control strains of streptococci were made with the serum from Normal Horse and from
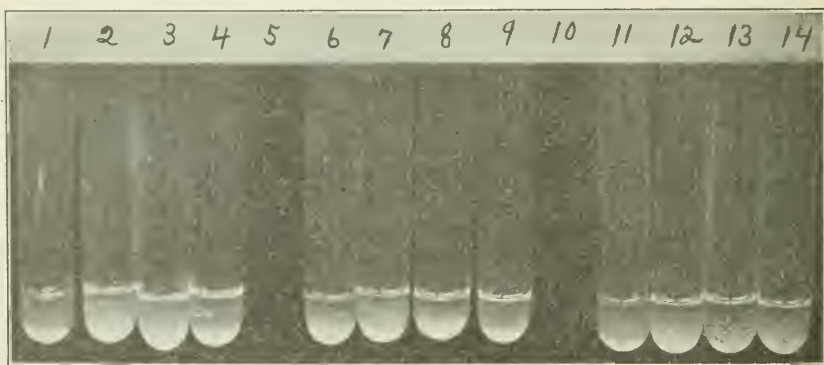
Fig. 5.—Agglutination of a strain from the tonsil in human poliomyelitis by the serum of Horse 1 and 3. Tubes 1-4 contained normal horse serum, 6-9 immune serums Horse 1 and 11-14 immune serums Horse 3. Note absence of agglutination in normal horse serum. Increasing agglutination up to 1:100 in the immune serums and its absence in the 1:1000 dilution.

Horse 1; 12 of these tests included the serum from Horse 3. The strains were isolated from a wide range of diseases or conditions, including 2 strains from meningitis. The length of time since the isolation of the different strains varied a great deal. Some were kept under the same conditions as the poliomyelitis strains from the time they were isolated until they were used in the experiment. Most of the strains had been kept on blood agar.

Table 9 gives the incidence of the upper limit of agglutination and the incidence of no agglutination of these control strains with the serum from Normal Horse, Horse 1, and Horse 3. The agglutinating power of the immune horse serum toward these strains is only slightly higher

TABLE 9

TOTAL INCIDENCE OF THE UPPER LIMIT OF AGGLUTINATION OF CONTROL STRAINS
WITH THE SERUM FROM NORMAL HORSE, HORSE 1, AND HORSE 3,
AND THE TOTAL INCIDENCE OF NO AGGLUTINATION

Dilutions of Serum	Serum from		
	Normal Horse, Percentage	Horse 1, Percentage	Horse 3, Percentage
1 : 1	40	22	17
1 : 10	35	24	42
1 : 100	3	16	17
1 : 1000	0	9	0
1 : 10,000	0	2	0
1 : 100,000	0	0	0
1 : 1,000,000	0	0	0
No agglutination	20	28	25

than that of the normal horse serum, which is in sharp contrast to the results obtained with the pleomorphic streptococcus. The few strains which were agglutinated in high dilutions by the immune serum from Horse 1 were usually agglutinated by normal horse serum in low dilutions.

These experiments were controlled in still another way. The agglutinating power of serum from horses immunized with pneumococci Types I and II, and of antistreptococcus and antimeningococcus serum was tested.* In Table 10 are given the results of a number of experiments made at the same time in which there were tested the agglutinating power of the antipoliomyelitis serums, antipneumococcus serums Types I and II, antimeningococcus and antistreptococcus serums, over the pleomorphic streptococcus and over control strains. Six antigens from human strains and 2 from monkey strains, together with 2 controls—a pneumococcus Type I and a hemolytic streptococcus (257)—were used. The strains from human poliomyelitis were agglutinated specifically by the serum from Horse 1 and Horse 3. It is of interest to note that after the 2 human strains (722 and 714) had been passed through animals, they became less agglutinable by the serum from Horse 1 and Horse 3 and specifically more agglutinable to antipneumococcus serum Type II. The amount of agglutination with the latter, however, was not as great as with the antipoliomyelitis serums.

The results with the monkey strains (Monkey 126.3, from the cord, and Monkey 126.4, from the brain) are of interest (Table 10). Both produced green colonies on blood agar. Monkey 126 was paralyzed

TABLE 10

Agglutination of the Pleomorphic Streptococcus and Control Strains by Antipoliomyelitis, Antipneumcoccus, Antimeningococcus and Antistreptococcus Serum

Strain	Dilutions of Serum	Serum						
		Normal Horse	Antipoliomyelitis		Antipneumococcus		Anti-meningo-coccus	Anti-strepto-coccus
			Horse 1	Horse 3	Type I	Type II		
722.8 (Brain)	1:1	+	+++	+++	+++	+++	++	+++
	1:10	+	++++	++++	++	+++	+	++
	1:100	0	++++	++++	+	++	0	++
	1:1000	0	+	+	0	0	0	0
	1:10,000	0	0	+	0	0	0	0
	1:100,000	0	0	+				
	1:1,000,000	0	0	0				
722³.3 (Brain) (R1010)	1:1	++	+++	++++	++	+++	++	+++
	1:10	++	+++	++++	+	++++	+	+++
	1:100	0	++++	++++	0	+++	0	+
	1:1000	0	++	++	0	0	0	0
	1:10,000	0	0	0	0	0	0	0
	1:100,000	0	0	0				
	1:1,000,000	0	0	0				
714.3 (Brain)	1:1	+++	++c	+++	++	+++		
	1:10	++	++	++++	++	++		
	1:100	0	++	++++	0	0		
	1:1000	0	+	++	0	0		
	1:10,000	0	+	+	0	0		
	1:100,000	0	0	0				
	1:1,000,000	0	0	0				
714⁵.2 (Brain) (M10)	1:1	+++	++	+++	+++	+++		
	1:10	+++	++	+++	++	+++		
	1:100	0	++++	+++	0	++		
	1:1000	0	++	0	0	0		
	1:10,000	0	0	0	0	0		
	1:100,000	0	0	0				
	1:1,000,000	0						
729 (Tonsil pus)	1:1	+	++++	+++	+	+	+	+
	1:10	+	+++	++	0	++	0	0
	1:100	0	++	0	0	0	0	0
	1:1000	0	0	0	0	0	0	0
	1:10,000	0	0	0	0	0	0	0
	1:100,000	0	0	0				
	1:1,000,000	0	0	0				
734 (Tonsil pus)	1:1	++	++c	+++	++	++	++	+
	1:10	0	+++	+++	++	+	++	++
	1:100	0	+++	++	0	+	0	++
	1:1000	0	++	+	0	0	0	0
	1:10,000	0	0	0	0	0	0	0
	1:100,000	0	0	0	0			
	1:1,000,000	0	0	0				
M126.3 (Cord)	1:1	++	+	+				
	1:10	+	++	+++				
	1:100	0	+++	+++				
	1:1000	0	0	0				
	1:10,000	0	0	0				
	1:100,000		0	0				
M126.4 (Brain) Green-producing streptococcus	1:1	+	+	+	+	0		
	1:10	0	0	0	0	0		
	1:100	0	0	0	0	0		
	1:1000	0	0	0	0	0		
	1:10,000	0	0	0	0	0		
	1:100,000	0	0	0	0	0		
	1:1,000,000							
Hemolytic streptococcus 257	1:1	0	+	+++	0	++	0	+++
	1:10	0	+	0	0	0	0	0
	1:100	0	0	0	0	0	0	0
	1:1000	0	0	0	0	0	0	0
	1:10,000	0	0	0	0	0	0	0
	1:100,000	0	0	0				
	1:1,000,000	0	0	0				
Pneumococcus Type I	1:1	0	0		0	+		
	1:10	0	0		−	−		
	1:100	0	0		+++	0		
	1:1000	0	0		+	0		
	1:10,000	0	0		0	0		
	1:100,000							
	1:1,000,000							

with virus. At necropsy some time after death, in addition to the typical findings of poliomyelitis, marked ulcerative colitis was found. Besides the green-producing strains a hemolytic streptococcus and colon bacillus were isolated from the nervous system. The green-producing strain from the cord as shown was agglutinated in Table 10, while the one from the brain was not. The former showed short chains, diplococci and single cocci of varying size in fluid cultures and should be regarded as the specific streptococcus. The latter showed long chains of elongated diplococci with no small forms and should be regarded as an accidental invader. The possibility, however, that the latter lost the specific agglutinating property must be considered.

TABLE 11

Total Incidence of the Upper Limit of Agglutination of Poliomyelitis Strains (Human and Monkey) by Serum from Horses Immunized with Pneumococci, Meningococci, and Streptococci, and the Total Incidence of No Agglutination

Dilutions of Serum	Serum
	Antipneumococcus Antimeningococcus Antistreptococcus
	Percentage
1 : 1..	19
1 : 10...	38
1 : 100..	32
1 : 1000...	0
1 : 10,000...	1
No agglutination...	9

In Table 11 is given the incidence of the upper limit of agglutination and of no agglutination of the poliomyelitis strains with antipneumococcus, antimeningococcus and antistreptococcus horse serum. Altogether 77 agglutination tests were made with strains from human and monkey sources. A comparison of the figures in Table 11 with the figures in Tables 6 and 7 giving the results of the agglutination of these strains with normal horse serum shows that the agglutinating power of these serums was only slightly higher than that of normal horse serum.

In Table 12 is shown the agglutinating power of normal and immune horse serum and human and monkey serum over a few strains of the pleomorphic streptococcus. Strain 714.3 was isolated a short time previous to this experiment from the dried brain of a patient with typical poliomyelitis. Strain $714^3.3$ was isolated from the preserved brain substance of Rabbit 1010, being in the third animal passage. Strain

TABLE 12

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS BY NORMAL AND IMMUNE HORSE, HUMAN, AND MONKEY SERUM

Strain	Dilutions of Serum	Horse Serum		Human Serum					
		Normal	Immune Horse 1	Normal E	Immune 833	Normal F	Immune 834	Normal D	Immune 831
714.3 (Brain)	1:1	+++	++c	0	+	0	++	++	++
	1:10	+	+++	0	++	0	++	+	++
	1:100	0	++++	0	+	0	0	0	++
	1:1000	0	++++	0	0	0	0	0	+
	1:10,000	0	++++	0	+	0	+	0	+
	1:100,000	0	+++	0	++	0	0	0	0
	1:1,000,000	0	+	0	0	0	0	0	0
714³.3 (Brain) (R1000)	1:1	+++	++c	++	++c	++	++c		
	1:10	++	+++	0	+c	+	++c		
	1:100	0	++++	0	++	0	+		
	1:1000	0	++++	0	++	0	+		
	1:10,000	0	+++	0	++	0	+		
	1:100,000	0	+	0	+	0	+		
	1:1,000,000	0	+	0	0	0	0		
707³.2 (Cord) (P339)	1:1	++	+++	+	++c	0	+c	0	+c
	1:10	++	++	0	++c	0	+c	0	++
	1:100	0	++	0	+	0	0	0	++
	1:1000	0	+++	0	+	0	0	0	+
	1:10,000	0	+++	0	0	0	0	0	+
	1:100,000	0	++	0	0	0	0	0	0
	1:1,000,000	0	++	0	0	0	0	0	0
M97.2 (Brain)	1:1	+++	+++	+	+++	+	++	+	+c
	1:10	++	++++	+	++	0	++	0	0
	1:100	0	++++	0	++	0	+	0	+
	1:1000	0	++	0	0	0	++	0	+
	1:10,000	0	+	0	+	0	++	0	0
	1:100.000	0	0		+		++		0
	1:1,000,000	0	0				0		0

Monkey Serum

Normal 1	Immune M52	Normal M121	Immune M24	Normal 3	Immune M85	Normal 1	Immune M97
+	++++	0	++				
0	0	0	+++				
0	+	0	++				
0	0	0	+				
0	0	0	0				
+	+++	0	+++	0	+++	+	+++
+	+	0	+	0	++	0	++++
0	0	0	0	0	0	0	+++
0	0	0	0	0	0	0	+
0	0	0	0	0	0	0	0
+	+++						
+	++						
0	+						
0	+						
0	+						
+	+++			0	+	0	+
+	+++			0	+	0	++
0	0			0	0	0	++
0	0			0	0	0	+
0	0			0	0	0	0

707³.2 was isolated August 19 from the fresh cord of a patient and was preserved in an anaerobic culture after 2 animal passages. The strain, Monkey 97, was recently isolated from the brain of a monkey paralyzed with virus. The results in the latter were similar to those shown in Figure 6.

Judging from the agglutinating power of the normal horse serum over these strains it is evident that they were in a relatively agglutinable condition, and hence favorable for detecting differences in the agglutinin content of normal serum and of the serum of patients and monkeys with poliomyelitis. It will be noted that the agglutination by the serum of normal persons and normal monkeys if present at all is
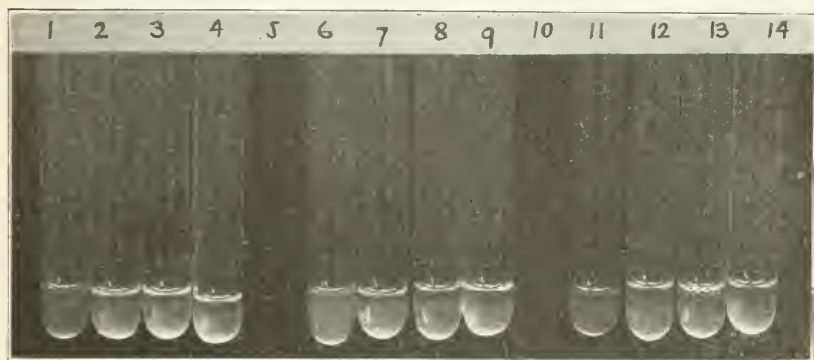
Fig. 6.—Agglutination of monkey strain (M 49.4) with human serum. Tubes 1-4 contained normal human serum. Tubes 6-9 and 11-14 contained the serum from 2 individuals (828 and 846) who had recovered from severe attacks of poliomyelitis. The control serum was obtained on the same day as the immune serums from an individual of approximately the same age. Note the absence of agglutination in the normal serum and the agglutination in the 1:1 and 1:10 dilutions of the immune serums.

slight; that it occurs only in low dilutions and is about equal. These serums, however, vary in their agglutinating power over the different strains. The ages of the normal and immune persons in the parallel columns were about the same, and the serums were obtained on the same day. The 3 patients (Cases 833, 834, and 831) were children in New York who had recovered from severe attacks of poliomyelitis with marked residual paralysis.* The agglutinating power of these serums, while not equally great over the different strains, was greater than the corresponding normal control in each instance, and in some instances was evident in high dilutions. The occurrence of increased clouding in

* We are indebted to Dr. George W. Wheeler and Dr. E. D. Ebright of New York, for the serum of these and other cases.

the low dilutions in some of these immune serums is in accord with the observations of the immune horse serums.

Monkey 52 was injected with a culture (in the second generation) of the pleomorphic streptococcus isolated from the brain of a poliomyelitic monkey (Monkey 34). Its serum agglutinated these strains markedly.

Monkey 24 was bled 4 months after it had recovered, with marked residual paralysis, from typical poliomyelitis following injection of virus.

Monkeys 85 and 97 were bled when nearly dead, two days after the onset of typical attacks of poliomyelitis, paralysis beginning six and ten days respectively, after intracerebral injection of virus.

The agglutinating power of the serum of these virus-immune monkeys was greater than the corresponding normal control in each

TABLE 13

AGGLUTINATION OF THE PLEOMORPHIC STREPTOCOCCUS BY THE SERUM OF NORMAL, VIRUS IMMUNE, AND CULTURE IMMUNE MONKEYS

Strains	Dilutions of Serum	Serum						
		Normal Monkey			Monkey 21	Monkey 24	Monkey 19	Monkey 14
		A	B	C				
714.3 (Brain)	1 : 1	0	0	+	++	+++	++	
	1 : 10	0	0	0	+	+++	++	
	1 : 50	0	0	0	+	++	+	
	1 : 250	0	0	0	0	0	+	
	1 : 1250	0	0	0	0	0	0	
M85.3 (Brain)	1 : 1	0	0	0	+	+++	+	
	1 : 10	+	0	0	++	++	++	
	1 : 50	+	0	0	+++	++	+++	
	1 : 250	0	0	0	+	++	+	
	1 : 1250	0	0	0	0	+	0	
Bryan (Control)	1 : 1	0	0	0	0	0	0	
	1 : 10	0	0	0	0	0	0	
	1 : 50	0	0	0	0	0	0	
	1 : 250	0	0	0	0	0	0	
	1 : 1250	0	0	0	0	0	0	
276 (Control)	1 : 1	0	0	0	0	0	0	
	1 : 10	0	0	0	0	0	0	
	1 : 50	0	0	0	0	0	0	
	1 : 250	0	0	0	0	0	0	
	1 : 1250	0	0	0	0	0	0	
721^2.3 (Cord) (P444) (Brain)	1 : 1			0	++	++	+	+
	1 : 10			0	++	++	+	++
	1 : 100			0	+	++	+	+
	1 : 1000			0	+	+	0	0
	1 : 10,000			0	0	0	0	0
M49.4 (Brain)	1 : 1			++	+++	++	+	+++
	1 : 10			0	+++	+++	++	+++
	1 : 100			0	++	++	+	0
	1 : 1000			0	+	+	0	0
	1 : 10,000			0	0	0	0	0

instance. Exactly comparable results are shown in Table 13 with still other strains and serums.

Monkeys 21 and 24 had recovered from attacks of poliomyelitis following injection of virus. Monkey 19 had been rendered resistant to virus by intracerebral injection of cultures of the pleomorphic streptococcus, and Monkey 14 by virus derived from culture (Fig. 7).

The strain, Monkey 85.3, was recently isolated from the brain of a poliomyelitic monkey; the strain, Monkey 49.4, was isolated some time previously from the filtrate of the brain emulsion of a poliomyelitic monkey and was preserved in a deep stab culture. Strain $721^3.3$ was
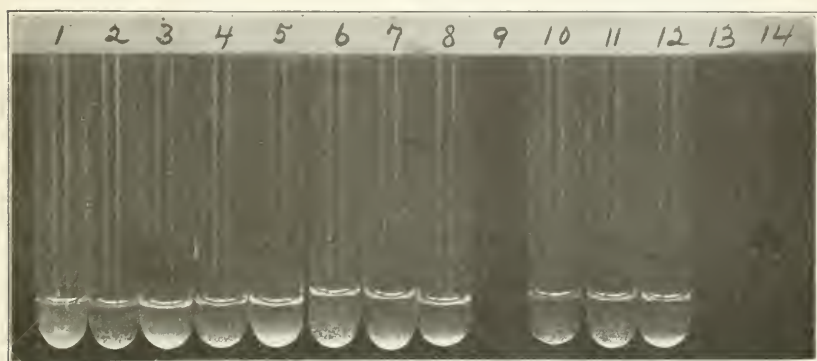
Fig. 7.—Agglutination of the pleomorphic streptococcus from poliomyelitis in the monkey (M. 49.4) with the serum of Monkey 21 paralyzed with virus, and the serum of Monkey 14 which resisted virus following intracerebral injection of virus from culture. Tubes 1, 3, 5 and 7 contained normal monkey serum, Tubes 2, 4, 6 and 8 contained immune serum Monkey 21, and Tubes 10, 11, and 12 contained immune serum Monkey 14. Note the complete absence of agglutination by the normal serum above the first or 1:1 dilution and the marked agglutination by the immune serums. The readings of this experiment are shown in Table 13.

isolated from the cord of a case of human poliomyelitis; it produced paralysis in a guinea-pig and was preserved in the brain of this animal until shortly before the antigen was prepared. The antigen 714.3 was the same as that used in the experiments in Table 2. The strains marked Bryan, and 276, used as controls, were from endocarditis and herpes zoster, respectively. These were not agglutinated by any of the serums. The serum from the virus-immune monkeys agglutinated all the poliomyelitic strains to a greater degree than any of the normal monkey serums. The point of special interest, however, is the fact that the serums from 2 monkeys (19 and 14) rendered resistant to virus by injection of culture of the pleomorphic streptococcus without apparently having had poliomyelitis, agglutinated these strains. In

TABLE 14

Total Incidence of the Upper Limit of Agglutination of Poliomyelitis Strains
by Normal and Immune Human Serum, and the Total Incidence
of No Agglutination

Dilutions of Serum	Serum	
	Normal, Percentage	Immune, Percentage
1 : 1	10	12
1 : 10	21	29
1 : 100	12	23
1 : 1000	0	12
1 : 10,000	0	5
1 : 100,000	0	1
1 : 1,000,000	0	1
No agglutination	57	18
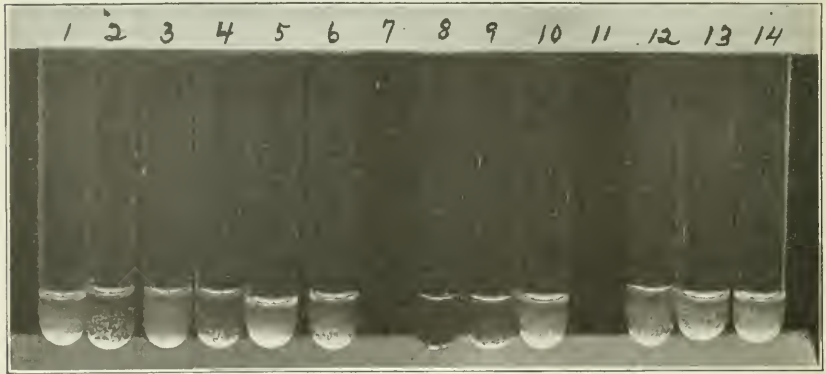
Fig. 8.—Agglutination of the pleomorphic streptococcus from poliomyelitis in the monkey (M 49.4) with the serum of monkeys paralyzed with virus. Tubes 1, 3, and 5 contained normal monkey serum, Tubes 2, 4, and 6 immune serum Monkey 53, Tubes 8, 9, and 10 immune serum Monkey 105, and Tubes 12, 13 and 14 immune serum Monkey 92. The serum of immune Monkeys 53, 105 and 92 was obtained 14, 8 and 16 days, respectively, after onset of severe attacks of poliomyelitis. All were given intravenous injections of serum from Horse 1 after paralysis had begun and all recovered.

addition to these experiments, the serums from Monkeys 21 and 19 together with the serums from 3 normal monkeys were tested against 1 other strain from human poliomyelitis (707) and 1 other strain from monkey poliomyelitis (Monkey 97). Agglutination occurred with the immune serum but not with the normal.

In Table 14 is given the incidence of the upper limit of agglutination of poliomyelitis strains by normal and immune human serum and the total incidence of no agglutination. These results were obtained in experiments in which 27 antigens were tested with the serums from 41 normal persons, from 2 persons having Streptococcus viridans infec-

tion, and from 27 poliomyelitis patients. Eighteen of the antigens were prepared from 13 human strains, and 9 from 4 monkey strains. Altogether there were made 137 agglutination tests with normal human serum and 146 with the serum from patients who had recovered from attacks of poliomyelitis. The upper limit of agglutination in these immune serums does not average as high as in the serums of horses hyperimmunized with these strains, but is sufficiently higher than in the normal controls to be of significance. The incidence of no agglutination was significantly less in the immune than in the normal serum. The agglutinating power of the serums from the 2 patients having Streptococcus viridans infections was no higher than that of some of the
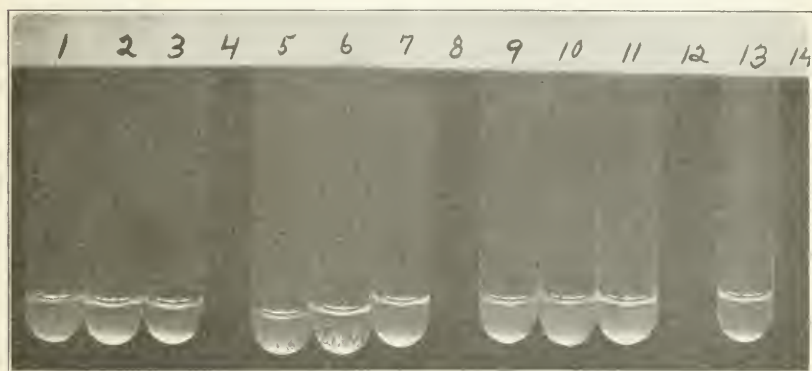
Fig. 9.—Agglutination of human strain after 5 animal passages (714^5.2) by monkey serum. Tubes 1-3 contained normal monkey serum (151). Note the slight agglutination in the 1: 1 dilution. Tubes 5-7 contained the serum of Monkey 144 paralyzed with virus. Note the marked agglutination in the 1: 1 and 1: 10 dilutions. Tube 13 was the salt solution control. Tubes 9-11 contained the serum of Monkey 144 paralyzed with virus and a suspension of a streptococcus from the brain of a monkey which died with poliomyelitis and ulcerative colitis.

normal serums. The agglutinating power of 4 of the normal human serums over some of these strains was nearly as marked as that of some of the immune serums.

All the normal individuals whose serum was used in these experiments were from regions where poliomyelitis occurred in epidemic form during the summer of 1916; 2 of them had been in close contact with the disease in man and animal. Four of the 25 poliomyelitis patients whose serum was tested were sporadic cases; the others occurred during the epidemic in the summer of 1916. Parallel agglutinations were obtained with the serum from the sporadic and epidemic cases, with the exception of the 1 sporadic case previously reported, the serum from which did not agglutinate these strains.

TABLE 15

Total Incidence of the Upper Limit of Agglutination of Human and Monkey
Poliomyelitis Strains by Normal and Immune Monkey Serum,
and the Total Incidence of No Agglutination

Dilutions of Serum	Serum from		
	Normal Monkeys, Percentage	Virus Immune Monkeys, Percentage	Culture Immune Monkeys, Percentage
1 : 1	6	2	7
1 : 10	21	33	32
1 : 100	9	20	16
1 : 1000	3	16	16
1 : 10,000	0	5	12
No agglutination	61	25	18

Table 15 gives a summary of the results obtained with the serums
from normal and immune monkeys over strains from human and
monkey poliomyelitis (Figs. 8 and 9). Altogether 30 antigens were
used: eighteen from 18 human strains, 10 before and 8 after, 1-4 animal
passages, and 12 from 10 monkey strains before animal passage. The
serum from 27 normal monkeys was used in a total of 152 agglutination
tests; the serum from 27 virus-immune monkeys in 200 tests; and the
serum from 14 monkeys immunized with cultures of the pleomorphic
streptococcus or with emulsions of brain or cord of monkeys that had
been injected with aerobic cultures in 57 tests.

The results correspond very closely to those obtained with normal
and immune human serums. Some of the culture-immune monkeys
appeared to have had abortive attacks of poliomyelitis and the serums
of 2 had the power to neutralize virus in the test tube.[2]

TABLE 16

Total Incidence of the Upper Limit of Agglutination of Control Strains by
Normal and Immune Human and Monkey Serum and the
Total Incidence of No Agglutination

Dilutions of Serum	Serum	
	Normal, Percentage	Immune, Percentage
1 : 1	26	2
1 : 10	11	35
1 : 100	18	2
1 : 1000	0	4
1 : 10,000	0	0
1 : 100,000	0	0
1 : 1,000,000	0	0
No agglutination	46	58

In Table 16 is given a summary of the agglutinating power of normal and immune human and monkey serum over control strains of streptococci. In these experiments there were used altogether 18 antigens prepared from 15 strains of streptococci or pneumococci from sources other than poliomyelitis. Thirty-five tests were made with normal human and monkey serum and 52 with immune human and monkey serum. The upper limit of agglutination and the incidence of no agglutination in the normal and immune serums is very nearly alike and corresponds closely to the results obtained with normal human and normal monkey serums over the poliomyelitis strains.

The agglutinating power of the serum from Horse 1 and Horse 3 was tested over 7 strains isolated from the nervous system in poliomyelitis, kindly sent me by Dr. Baldwin Lucke of Philadelphia. These had been cultivated continuously aerobically on ascites dextrose-agar and blood-agar slants since isolated during the summer of 1916. They resembled very closely the strains with which we have worked. Three were of the micrococcus type and 4 of the streptococcus type at the time the antigens for agglutination tests were prepared. Five of the strains were agglutinated markedly and to about the same degree by the serum from Horse 1 and Horse 3; 2 were not agglutinated. The upper limit of agglutination was 1 : 1000 or above in 13 out of a total of 29 tests. In the normal horse serum the upper limit was never above 1 : 100 and usually occurred only in dilutions of 1 : 1 or 1 : 10 if at all. The serum from 2 persons who had recovered from poliomyelitis agglutinated specifically 3 of 6 of these strains.

The results of the agglutination tests with a strain isolated from the pons in human poliomyelitis, kindly sent me by Dr. Meyer Solis-Cohen of Philadelphia, are also of interest. He has found a high opsonic index toward this strain in the serum of patients who have recovered from poliomyelitis. The serum from Horse 1 and Horse 3 agglutinated this strain specifically in high dilutions, on repeated occasions. It was also agglutinated by the serum of 8 virus-immune monkeys.

SUMMARY OF EXPERIMENTS ON THE AGGLUTINATION OF STRAINS ISOLATED FROM THE TONSIL

It was difficult to isolate the pleomorphic streptococcus from the tonsil and to differentiate it unmistakably from the green-producing streptococcus normally present in tonsils. It was thought worth while therefore to make agglutination tests with strains from tonsils which from their morphologic and cultural characteristics or pathogenic power were considered of etiologic importance when isolated. Altogether 41

agglutination tests with the serum from Horse 1 and Horse 3 were made with 28 antigens prepared from 16 tonsil strains, 20 before animal passage and 8 after 1 or 2 animal passages. Most of the strains had been grown on aerobic blood-agar slants since they were isolated in the summer of 1916 and were in the fourth to the tenth culture generation. Some were kept in deep stabs of ascites tissue fluid and a few in sealed pipets containing the brain substance of paralyzed animals.

Agglutination was more marked with the immune serum of Horse 1 than with the normal horse serum in all but 9 tests. The negative tests included 5 antigens, 4 of which were made from a tonsil strain isolated 6 weeks after the onset of attack and after 1 animal passage. Two tests were made with a strain cultivated aerobically on blood agar since isolation and 2 with strains obtained from the tonsils of 2 cases of sporadic anterior poliomyelitis some weeks after the attack. In these, negative results were to be expected. In only 1 test was the result contrary to expectations. In this instance the antigen was prepared from a strain from the brain of a cat which developed paralysis following intravenous injection of a culture made from the tonsil at the time of the attack.

Agglutination was more marked in the immune serum of Horse 3 than in the serum of Horse 1 in all but 3 tests (Fig. 5). The negative tests occurred with 3 antigens prepared from 3 strains cultivated continuously aerobically on blood-agar slants since isolation. In 2 instances, parallel tests were made with antigens from the same strains, 1 grown continuously on blood agar, the other in deep tissue agar stabs. In one, the upper limit of agglutination was 1 : 1000 by both serums with the antigen prepared from the stab cultures and 1 : 100 by both serums with the antigen prepared from the blood-agar culture. In the other instance the antigens from both types of cultures were agglutinated alike, the upper limit being 1 : 100. No agglutination whatsoever occurred with the normal serum 10 times, whereas only once did the immune serum fail to agglutinate.

Some of the more sensitive strains were selected for agglutination tests with immune human serum and immune monkey serum. Agglutination above that with the respective normal serum was obtained in 5 out of 8 experiments with immune human serum and in 8 out of 14 experiments with immune monkey serum. Immune human and immune monkey serum had no agglutinating power over staphylococci, diphtheroid bacilli and colon bacilli isolated from the nervous system of patients and monkeys.

DEVELOPMENT OF AGGLUTININS IN THE SERUM OF MONKEYS

During the course of these experiments it was found that the agglutinin content of the immune serums from horse, man and monkey did not become appreciably less for some weeks if kept in the ice chest. In most of the experiments the control normal serum was obtained on the same day as the immune serum. In only 2 instances was the serum of the same monkey used as normal and immune. It was desirable therefore to test the agglutinin content of the serum of monkeys previous to injection and at intervals following injection of active virus. If the development of agglutinin has significance, agglutination should occur with strains from human sources and with homologous as well as heterogeneous strains isolated from poliomyelitic monkeys.

In Table 17 is given a summary of an experiment in which these conditions were fulfilled. There were used as antigens 1 strain from brain and tonsil (729), 1 from the pons from a case of typical poliomyelitis in man (899), and 1 from the cord of a monkey (Monkey 145) paralyzed with the virus which we had used in connection with the above experiments (heterogeneous strain), and 1 from a monkey (Monkey 148) paralyzed with the virus obtained recently from the Public Health Laboratories and which was used to inject the monkeys in this experiment (homologous strain). Several control strains were included, the results of only one ($622^{35}.7$) are given in the table. The poliomyelitis antigens were previously found to be agglutinated specifically in high dilution by the serum from the immune horses, and for this reason were selected for the experiment.

Monkeys 147 and 150 (given 1 injection of immune serum on April 21) were first used as controls. All were bled every other day from April 18 to April 29. The blood was allowed to clot, placed in the ice chest, and the serum drawn off the following day. All serums were kept in the ice chest until used.

On April 21, Monkey 148 was injected intracerebrally with 1 c.c. of a 5% emulsion of the glycerinated virus. Paralysis was marked in both arms April 27, and April 29 the animal was completely prostrate. The agglutination experiment with the serum obtained up to April 29 was performed May 10.

On May 2, Monkeys 147 and 150 were bled again and then injected intracerebrally with 1.5 c.c. of a 5% emulsion of the glycerinated virus. Monkey 147 became paralyzed May 9 and was prostrate May 14. The results on the dates given in the table indicate accurately those obtained

TABLE 17

The Development of Agglutinins for the Pleomorphic Streptococcus in the Serum of Monkeys with Paralyzed Virus

Strain	Dilutions of Serum	Serum from									
		Monkey 147				Monkey 148			Monkey 150			
		Normal			Immune	Normal		Immune	Normal			Immune
		4/18	4/29	5/2	5/14	4/18	4/20	4/29	4/18	4/29	5/2	5/14
729.9 (Cord)	1 : 1	—	—	—	0	—	—	—	—	—	—	+++
	1 : 10	0	0	0	+	+	+	++	+	+	0	++
	1 : 50	0	0	0	0	0	0	+	0	0	0	0
	1 : 250	0	0	0	0	0	0	0	0	0	0	0
	1 : 1250	0	0	0	0	0	0	0	0	0	0	0
	1 : 6250	0	0	0	0	0	0	0	0	0	0	0
	1 : 31,250	0										
729 (Tonsil pus)	1 : 1	—	—	—	0	—	—	—	—	—	—	+
	1 : 10	0	0	0	+	0	0	+	0	+	0	0
	1 : 50	0	0	0	+	0	0	+	0	0	0	0
	1 : 250	0	0	0	0	0	0	0	0	0	0	0
	1 : 1250	0	0	0	0	0	0	0	0	0	0	0
	1 : 6250	0	0	0	0	0	0	0	0	0	0	0
899 (Pons)	1 : 1	—	—	—	++	—	—	—	—	—	—	+++
	1 : 10	+	0	0	++	++	0	+++	+	+	0	+++
	1 : 50	0	0	0	+	0	0	++	+	+	0	++
	1 : 250	0	0	0	0	0	0	+	0	0	0	+
	1 : 1250	0	0	0	0	0	0	0	0	0	0	0
	1 : 6250	0	0	0	0	0	0	0	0	0	0	0
M145.3 (Cord)	1 : 1	—	—	—	0c	—	—	—	—	—	—	+c
	1 : 10	0	0	0	0	0	0	++	0	0	0	++
	1 : 50	0	0	0	0	0	0	+	0	0	0	+
	1 : 250	0	0	0	0	0	0	+	0	0	0	0
	1 : 1250	0	0	0	0	0	0	0	0	0	0	0
	1 : 6250	0	0	0	0	0	0	0	0	0	0	0
M148.2 (Cord)	1 : 1	—	—	—	+	—	—	—	—	—	—	++
	1 : 10	0	0	0	++	0	0	++	0	0	0	++
	1 : 50	0	0	0	0	0	0	+	0	0	0	+
	1 : 250	0	0	0	0	0	0	+	0	0	0	0
	1 : 1250	0	0	0	0	0	0	0	0	0	0	0
	1 : 6250	0	0	0	0	0	0	0	0	0	0	0
622^{35}.7 (Pneumo-coccus) Control	1 : 1			—	0						—	—
	1 : 10			0	0						0	0
	1 : 50			0	0						0	0
	1 : 250			0	0						0	0
	1 : 1250			0	0						0	0
	1 : 6250			0	0						0	0

in the other bleedings except that in Monkey 148 there was a distinct increased agglutinating power of the serum on April 27 over all the strains. This increase, however, was not as marked as on April 29. There was no demonstrable diminution in the agglutinating power of the serum due to age.

The agglutinin content of the serum was increased in all the monkeys that developed typical attacks of poliomyelitis. This was shown toward all the strains except in 2 instances. The increase in agglutinating power by the immune serum was no greater toward the homologous strain (Monkey 148) than toward the heterogeneous strains (729, 899 and Monkey 145). The serum of Monkey 148, however,

agglutinated the strain from this monkey slightly more markedly than did that of Monkeys 147 and 150. There was no increase in agglutination of the control pneumococcus strain. The cultures from the brain of Monkey 148 showed, in addition to the pleomorphic streptococcus, a hemolytic streptococcus which was not agglutinated by the serum from any of the bleedings of this monkey.

SUMMARY

The pleomorphic streptococcus isolated from the tonsil and central nervous system of human poliomyelitis and from the central nervous system of monkeys paralyzed with virus has marked antigenic properties.

The strains from both human and monkey poliomyelitis are cross-agglutinated in high dilution by the serum from horses hyperimmunized with human and monkey strains respectively, and in lower dilution by the serum of persons who have had poliomyelitis. Moreover the serum of monkeys acquires specific agglutinating power over these strains as they develop poliomyelitis following injection of virus. This agglutinating power of the serum following poliomyelitic attacks has been shown to persist for months, and hence cannot be regarded as due to mobilization of preformed antibodies or to nonspecific changes in the serum incident to fever etc. at the time of the attack. Streptococci and pneumococci from sources other than poliomyelitis are with few exceptions not agglutinated more by the antipoliomyelitis serums than by normal horse serum. A few of a large number of strains approached in agglutinability the pleomorphic streptococcus.

Normal human and normal monkey serum has little or no agglutinating power over the poliomyelitis strains or over control strains. The agglutinin content toward these strains of serum of persons and monkeys suffering from other streptococcus infections was no higher than of the respective normal serums. Poliomyelitic human and monkey serums showed no increase in agglutinating power over streptococci from sources other than poliomyelitis. Antipneumococcus, antimeningococcus and antistreptococcus horse serums do not agglutinate the poliomyelitic strains more than normal horse serum.

Judging by the results with normal horse serum the pleomorphic streptococcus is more easily agglutinated than green-producing streptococci and pneumococci from a wide range of sources. A method has thus been found which proves that the streptococcus, found so con-

stantly in poliomyelitis, is immunologically quite distinct when first isolated. There is a marked difference in the degree with which the various strains retain their specific agglutinability. Anaerobic cultivation tends to preserve this property; aerobic cultivation tends to destroy it. It may be lost by either method without noticeable changes in morphology or cultural characteristics, but usually these changes occur simultaneously. Some strains retain the specific agglutinating condition through many culture generations. In some instances it may be lost suddenly even during one subculture. The specific agglutinating condition was preserved for months in the dried brain substance of human cases and in brain substance in sealed pipets of animals showing paralysis.

By means of agglutination experiments it has been possible to differentiate the pleomorphic streptococcus from green-producing streptococci isolated occasionally from the central nervous system of uninoculated and inoculated animals. The latter may be regarded as antemortem or postmortem invaders.

The results support the view that the elective localizing power of the pleomorphic streptococcus as first demonstrated by Rosenow, Towne and Wheeler[4] has significance and that it in some way bears etiologic relationship to epidemic poliomyelitis.

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3. Rosenow, E. C.: The Treatment of Epidemic Poliomyelitis with Immune Horse Serum. Preliminary Report. Jour. Am. Med. Assn., 1917, 69, pp. 1074-1075.

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REPORT ON THE TREATMENT OF FIFTY-EIGHT CASES OF EPIDEMIC POLIOMYELITIS WITH IMMUNE HORSE SERUM

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It is my purpose in this paper to report in detail the results obtained from the serum treatment of 58 cases of poliomyelitis which occurred in Davenport, Iowa, and the surrounding locality during the latter part of the summer and in the autumn of 1917.

The details of the immunization of the horse whose serum was used, the effect of this immune serum on the virus of poliomyelitis in vitro, its protection and curative power against experimental poliomyelitis in the monkey, its agglutinin and other antibody content,[8] and its effect in 44 cases of human poliomyelitis, have already been published.[9] Suffice it to point out here that the horse was injected at intervals (from Nov. 2, 1916, to May 1, 1917) with increasing doses of pure cultures of the pleomorphic streptococcus isolated a short time previously from the central nervous system of monkeys paralyzed with virus. Altogether the growth from 42,200 c.c. of broth were injected.

DIAGNOSIS

The diagnosis was made in the cases treated on the combined clinical and spinal fluid findings. All patients presenting symptoms of an acute infection with unusual evidence of involvement of the central nervous system were subjected to spinal puncture. If the sipnal fluid was under increased pressure, if it was clear or only slightly turbid, and if it contained an abnormal number of cells—chiefly mononuclear —and an increased globulin content, as determined immediately at the bedside by a count and Noguchi's globulin test, the diagnosis of poliomyelitis was made and the serum was given at once.

In all cases there was more or less fever, often high at the onset, which usually began without a chill. Gastro-intestinal symptoms were pronounced in the epidemic and were frequently initial, especially in children. Vomiting and diarrhea occurred in most instances and per-

sisted in some. At first the vomitus contained undigested food though later, in the severe cases, it contained large amounts of mucus and coffee-ground material. There was a great deal of mucus in the stools, and at times blood and bloody pus. The pulse rate was unusually rapid and out of all proportion to the evidence of general intoxication. Headache, irritability and restlessness, followed by abnormal apathy or drowsiness, tremor, twitchings of various muscles, pain in one or more extremities or in the back or neck, ataxia, retraction of the head, rigidity and tenderness of neck and spine, disturbed reflexes, and Kernig or Babinski signs, were the chief symptoms which usually preceded the onset of paralysis and led to the tentative diagnosis of poliomyelitis and immediate spinal puncture for further diagnostic tests.

TECHNIC OF TREATMENT

In making the spinal puncture a needle of the usual type but 2 cm. shorter, with a sharp, polished edge was used. In babies the puncture was made through the interspace, one, two or three spaces higher, depending on the age of the patient, than the one on the level of the crest of the ilium. A point just below and a little toward the side on which the patient was lying was selected for the puncture. The skin was sterilized with iodin or alcohol. The needle was inserted slowly through the skin and then carefully through the deeper layers, directing it slightly upward and aiming for the median line. The puncture was made just through the dura which can easily be felt. In this way trauma and pain were reduced to a minimum and the fluid was obtained without the admixture of blood. The fluid was made to flow slowly and only moderate amounts were withdrawn. The intravenous injections were made through a fine needle (20-23 caliber) with 10 or 20 c.c. syringes of the Lüer type. Superficial veins at the bend of the elbow, about the wrists, ankles, or dorsum of the hands were used chiefly. In babies the injections may be made in the jugular vein.

The serum used was obtained from the horse on May 16. It agglutinated homologous and heterologous strains of the pleomorphic streptococcus in dilution up to 1-100,000 and protected monkeys against virus. It was preserved in the icechest after adding 0.2% purified cresol. Aerobic and anaerobic cultures proved sterile on repeated occasions. Previous to administration it was usually activated with guinea-pig complement by adding 1 part of fresh guinea-pig serum to 9 parts of the serum, thoroughly mixing and incubating at 37 C. for

1 hour.* To facilitate slow injection and a more rapid diffusion in the blood, the activated serum was diluted with equal parts of 0.85% salt solution. The injections were made slowly intravenously, not later than 36 hours after activation. About 1 c.c. of serum or 2 c.c. of the diluted serum were injected per minute. The dose was varied according to the age of the patient and the severity of the symptoms. The dose as reported in my preliminary report was practically doubled later and with seemingly greater benefit. Inquiry as to whether or not the patient had had diphtheria or tetanus antitoxin or horse serum was made in each case. If any of these serums had been given the patient was first desensitized, provided his condition allowed it, by injecting a small amount (0.5 c.c.) subcutaneously or 0.25 c.c. intravenously, one-half to 2 hours previously. Babies up to 2 years of age were given from 5-15 c.c. of serum, that is, 12-30 c.c. of the diluted serum; children from 2-5 years, 10-20 c.c.; from 5-12 years, 15-30 c.c.; from 12 years up, 20-50 c.c. All patients with a positive diagnosis and in whom the disease was still active were given the serum treatment irrespective of the severity or type of the disease. The injections at first were usually given once in 24 hours. Later they were repeated in from 4, 8, 12, to 24 hours as necessary. The return of fever and high pulse rate after the initial drop which occurred commonly and the return of symptoms referable to the central nervous axis (irritability, twitchings, pain in extremities, rigidity of neck, etc.) after a primary disappearance or diminution, or the persistence of these symptoms, were considered indications for the giving of more serum.**

GROUP 1. CASES SHOWING NO PARALYSIS AT THE TIME OF SERUM TREATMENT

CASE 936.—K. K., a boy, aged 9 years. (Patient of Dr. W. Matthey, Davenport, Iowa.)

Sept. 1, 4 p. m.—The boy had had diarrhea 2 days before. Headache, restlessness, and fever began the previous day, and he was unable to sleep during the night. He had an attack of vomiting in the morning which persisted most of the day. There was tingling and a numb feeling in the hands. He was irritable and nervous, the head was retracted and the knee jerks were exaggerated. The throat was red and hyperemic; the tonsils were large, especially the right; the cervical gland outside the right tonsil was also enlarged. There was no evidence of weakness. The temperature was 101, the pulse 120. A spinal

puncture was made; the fluid was under moderate pressure and 15 c.c. were obtained. Cell count 60, globulin test ++. Ten c.c. of serum were given.

Sept. 2, 9 a. m.—The headache continued into the night; the temperature by midnight was 103 F. The headache and temperature then rapidly disappeared. There was no weakness and the child felt well.

This patient made a complete recovery without evidence of paralysis.

Case 944.—M. R., a boy, aged 11 years. (Patient of Dr. R. R. Kulp, Davenport, Iowa.)

Sept. 7.—There had been general irritability and restlessness with persistent headache and high fever ranging from 102.5-104 for 4 days previously. The child vomited 4 days before and complained of a sore throat, stiffness in the neck and severe pain in the back, especially when trying to get up. He was delirious part of the time at night. The throat and tonsils were unusually red, but there was no cryptic material and no pus was expressed. 7 p. m.—There was a moderate stiffness of the neck and back. Attempts at flexion caused pain. The left knee jerk was diminished, and the right exaggerated. There were no twitchings, but a moderate mental apathy. The temperature was 104.4. A spinal puncture was made; the fluid was under pressure and 15 c.c. were obtained. Cell count 16, globulin test +. Twelve c.c. of serum were given.

Sept. 8.—The white blood count was 18,000; Widal test negative; temperature 103 F. No paralysis could be made out. The general condition was about the same.

Sept. 9.—The temperature was 100; the child felt better and looked brighter but still complained of some stiffness of the neck. The pain in the back had disappeared. The right knee jerk was normal, the left was still somewhat sluggish.

Sept. 10.—He felt perfectly well and there was no paralysis.

Sept. 23.—A typical attack of serum disease occurred following overexertion the previous day.

Oct. 15.—The child was perfectly well. There was no paralysis at any time.

Case 950.—M. C., a girl, aged 2 years. (Patient of Dr. F. Neufeld, Davenport, Iowa.)

Sept. 12.—She felt well until 2 days previously in the afternoon, when she became unusually restless and later drowsy. She was very drowsy and sleepy the next day; slept most of the time with the head retracted. The mother became alarmed because of peculiar jerkings of the muscles of the arms, especially when the child was asleep. 8:30 a. m.—The temperature was 101.5. There were twitchings of the muscles of the hands, she was tremulous on getting up and ataxic in walking. She slept most of the time. 12 m.—The temperature was 101.5. The head was retracted, the neck stiff, and the knee jerks exaggerated. She was tremulous and there were twitchings of the muscles of the arms. No weakness was demonstrable. A spinal puncture was made and the fluid found under pressure. Cell count 150, globulin test +. Six c.c. of serum were given.

Sept. 13, 8 a. m.—She slept most of the night, with slight twitchings. The temperature was 98.8, the pulse 110.

Sept. 14.—She appeared well; the temperature was 98. She slept well all night and was bright and happy the next morning. No weakness was evident.

Oct. 15.—Attack of urticaria 1 week following injection of serum. Perfectly well.

Case 953.—N. W., a boy, aged 3 years; brother of Case 948. (Patient of Dr. F. Neufeld, Davenport, Iowa.)

Sept. 13.—The child was well until the previous night, when he seemed feverish; the temperature was 100.5. He vomited at 11 o'clock and then slept the balance of the night. He was drowsy all the next forenoon. 12 m.—There was a slight rigidity of the neck. The tonsils were hyperemic and large; no attempt was made to express pus. There was an enlarged lymph gland on either side just outside the tonsil. The right knee jerk was exaggerated, the left diminished. Kernig was evident on both sides. There was twitching of the muscles of the right hand and fingers. The child appeared abnormally drowsy. The temperature was 101.5, the pulse rapid. There was no weakness. 12:30 p. m.—A spinal puncture was made, with spurting of fluid and 10 c.c. were obtained. Cell count 18, globulin test +. Ten c.c. of serum were given. 1:30 p. m.—The child appeared brighter, was interested in surroundings, smiled and appeared quite normal. The rigidity of the neck was less marked. 7 p. m.—The patient was bright, talkative and alert. Supper was eaten with relish. The temperature was 101.

Sept. 14.—He appeared well. The temperature was 99. The knee jerks were normal. Rigidity of the neck and Kernig were absent. There was no weakness.

Oct. 15.—The child was perfectly well.

(Temperature and pulse curves, Chart 1.)

CASE 954.—A. J., a boy, aged 9 years. (Patient of Dr. F. Neufeld, Davenport, Iowa.)

Sept. 13.—He had had headache in the afternoon 4 days before, followed by pain in the back of the neck, and high fever. The next day he had pain in the abdomen followed by severe diarrhea a day later. He had no appetite. 4 p. m.— The temperature was 103, the pulse 134. The tonsils were large and pus was expressed from the left with slight pressure. They were diffusely red and there was 1 large lymph gland adjacent to each tonsil. The teeth were normal. The abdomen was diffusely tender on deep pressure. There was no tenderness at McBurney's point. The knee jerks were normal on the right side, but sluggish on the left; response was obtainable only on reinforcement. There was twitching of the hands and a slight ataxia in walking. The back and neck were held rather rigid and the Kernig sign was present. A spinal puncture was made; the fluid was under moderate pressure and 3 c.c. were obtained. Cell count 11, globulin test +. Twelve c.c. of serum were given.

Sept. 14, 8 a. m.—The temperature was 99.4. 6:30 p. m.—The temperature was 100.2 F., the pulse 112. The right knee jerk was normal, the left still weak. The child appeared well. The headache had disappeared; the stiffness of the neck and Kernig sign were absent, and he walked without difficulty. There was no ataxia or weakness. 9:10 p. m.—Five c.c. of serum were given.

Sept. 18.—The temperature was normal. There was no weakness and the boy appeared well.

Sept. 24.—The child had felt perfectly well until the morning when a typical mild attack of appendicitis developed, from which he recovered without surgical interference.

Oct. 15.—Recovery was complete.

CASE 956.—G. M., a boy, aged 5 years. (Patient of Dr. F. Neufeld, Davenport, Iowa.)

Sept. 13.—The patient was well until 4 o'clock in the afternoon, when he went into the house complaining of a severe headache. He became very ill shortly afterward and at 8:30 that evening was tremulous, vomited, and had severe diarrhea. The temperature was 103 F., the pulse 145. 12 p. m.—He was lying in bed with head retracted and was very dull mentally. He could be aroused but

would promptly fall back to sleep. Attempts at flexion of the head caused pain. The Kernig sign was elicited on both sides. The eyes were dull. He was tremulous and there were twitchings of various muscles. The tonsils were normal in size and the pharynx was normal. The knee jerk on the right side was exaggerated. There was no weakness. The child appeared extremely ill. He vomited a large amount of mucus streaked with blood. There was a marked diarrhea, the stools containing a large amount of mucus, some of it streaked with blood. The temperature was 103, the pulse 140. A spinal puncture was made; the fluid was under moderate pressure and 10 c.c. were obtained. Cell count 44, globulin test +. Ten c.c. of serum were given.

Sept. 14, 8:30 a. m.—The temperature was 101, the pulse 130. The child looked brighter. Some stiffness of the neck was still evident and there was slight Kernig. The left knee jerk was normal, the right somewhat diminished. He complained of pain in the abdomen. 6 p. m.—The temperature was 99.2, the
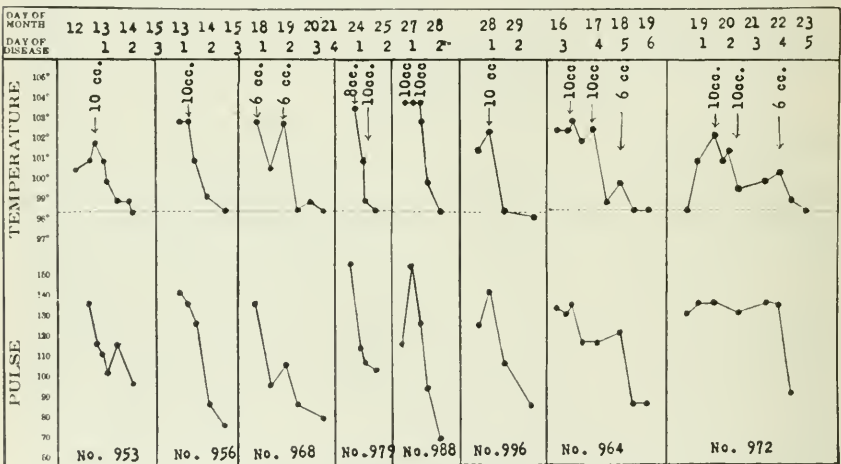
Chart 1. Temperature and pulse curves of patients showing no paralysis at the time of serum treatment.

pulse 90. He looked well and was hungry; the bowels were still loose. The neck was slightly rigid, the knee jerks were normal, and he walked without ataxia. No evident weakness. Five c.c. of serum were given.

Sept. 15.—The child appeared perfectly well. The temperature was 98.6 F., the pulse 80. There was no weakness.

Oct. 15.—Recovery was complete.

(Temperature and pulse curves, Chart 1.)

Case 962.—E. H., a young woman, aged 24 years. (Patient of Dr. L. F. Newburn, McCausland, Iowa.)

Sept. 15.—There had been malaise and she was generally below par for 3 or 4 days. She became extremely tired the day before, presumably from over-exertion. She had been unable to sleep the night before on account of restless-ness and pain in the abdomen. A number of loose bowel movements had occurred that day. She had severe headache in the afternoon. The temperature was 102 F., the pulse rapid. She was menstruating. 11:30 p. m.—The patient

had severe headache and pain in the back and felt very weak. There were twitchings of the muscles of the legs and a moderate rigidity of the neck. Flexion of the head caused pain. The knee jerks on both sides were very weak, barely obtainable even on reinforcement. The tonsils were large and there was a diffuse hyperemia of the throat and mucus in the nasopharynx. One large cavity was found in a left lower molar. The face was flushed. The temperature was 103.5, the pulse rapid. A spinal puncture was made; the fluid was under pressure and 20 c.c. were obtained. Cell count 23, globulin ++. Twelve and one-half c.c. of serum were given.

Sept. 16.—The physician found the patient sitting at the breakfast table. The headache had disappeared within 1 hour after the injection of the serum. The temperature was normal. There was no weakness.

'Oct. 14.—The patient was perfectly well.

CASE 964.—H. J., a girl, aged 4 years, sister of Case 972. (Patient of Dr. A. Grassau, Princeton, Iowa.)

Sept. 16.—The child was perfectly well until 2 days before, when she had fever and was cross and irritable. The nose was discharging. She seemed abnormally drowsy. The condition was much the same the day before but peculiar jerkings of the muscles had developed in the extremities. She complained of headache in the morning and had 6 attacks of convulsive jerkings of the extremities. She appeared confused mentally. 11:30 p. m.—The face was symmetrical, flushed, and she lay with head retracted. She was tremulous; ataxic, scarcely able to walk. Attempts to flex the head caused pain. She was very drowsy but could be aroused. The knee jerks were exaggerated, more on the right side than on the left. Kernig and Babinski signs existed on both sides and hyperesthesia was noted. The tonsils were large and red. Much mucus was found in the nasopharynx. There were 2 palpable lymph glands outside either tonsil. The thyroid was enlarged. There was marked ataxia, and peculiar spasms of the muscles of the arms and face. The child appeared very ill, was very tremulous and ataxic and could scarcely walk, but there was no weakness. Spinal puncture was made; the fluid was under pressure and slightly turbid and 10 c.c. were obtained. Cell count 211, globulin test +. Ten c.c. of serum were given.

Sept. 17, 6 p. m.—She lay quiet in bed, the face was flushed, and the head retracted. The stiffness of the neck was less marked. Kernig was absent. The tremor and twitching of the muscles had disappeared. The face was symmetrical. She was mentally brighter and there was no weakness. Ten c.c. of serum were given.

Sept. 18, 6 p. m.—She looked bright and appeared well. There was no retraction of the head. The pain and stiffness of the neck had disappeared. The knee jerks were normal and there was no rigidity of the neck and no weakness. Six c.c. of serum were given.

Sept. 19.—She appeared perfectly well and without demonstrable weakness.

Oct. 14.—The child was perfectly well.

(Temperature and pulse curves, Chart 1.)

CASE 966.—H. S., a boy, aged 3½ years. (Patient of Dr. E. O. Ficke, Davenport, Iowa.)

Sept. 17.—He had vomited three or four times 2 nights before and was restless all night. He was feverish and unusually drowsy the next day and complained of headache and pain in the back. There was looseness of the bowels. Marker tremor of various parts of the body was noted in the forenoon. The gait was ataxic. The knee jerks on the left side were exaggerated and on the

right side diminished. 5 p. m.—There was a moderately severe cough. The tonsils were large. There was one lymph gland palpable on either side of the neck adjacent to the tonsil and the thyroid was distinctly enlarged. The head was retracted and the neck was definitely stiff. Attempts to flex the head caused pain. There was a marked tremor of the muscles of the hands and legs, and the gait was ataxic. The knee jerk on the left side was exaggerated and on the right side diminished. The temperature was 103 F., the pulse 152. A spinal puncture was made, with spurting of fluid, and 12 c.c. were obtained. Cell count 27, globulin test + +. Ten c.c. of serum were given.

Sept. 18.—One hour after the injection of serum the previous day, the boy appeared more tremulous and the head more retracted. 8 p. m.—The temperature was 103.6 F., the pulse 148. Seven and one-half c.c. of serum were given.

Sept. 19, 10 a. m.—There was herpes of the lips and nostrils. The temperature was 98.6 F., the pulse 116. The rigidity of the neck and tremor of muscles had disappeared. The reflexes were normal. There was no weakness and the child looked well.

Oct. 15.—There was slight fever and cough for a number of days subsequently, but there was complete recovery without paralysis.

Case 968.—O. N., a girl, aged 1 year. (Patient of Dr. E. O. Picke, Davenport, Iowa.)

Sept. 18.—Onset with restlessness, looseness of bowels, nausea, and abnormal drowsiness. 8 p. m.—The temperature was 103, the pulse 140. The child cried when handled and did not want to be moved. The knee jerks were exaggerated, ataxia was marked, the head was retracted and the neck stiff. 11 p. m.—There was marked trembling of the extremities, ataxia, the head was retracted, the neck was stiff, and the knee jerks were exaggerated. The tonsils were large and moderately red and 2 follicles were filled with cheesy exudate. The larynx was diffusely hyperemic and there were palpable lymph glands outside of either tonsil. Seven teeth were found to be in process of eruption. Spinal puncture was made; the fluid was under increased pressure and 1.5 c.c. were obtained. Cell count 40, globulin test +. Six c.c. of serum were given.

Sept. 19, 10 p. m.—The patient slept all night and appeared brighter in the morning. She had felt well all day, had been up playing until about 5 o'clock. The tremor was absent and she walked without ataxia. The knee jerks were normal. There was no weakness. Six c.c. of serum were given.

Sept. 20.—The temperature was normal. She looked well and was bright and active. No weakness was noted.

Oct. 1.—The temperature was normal and she was perfectly well, up and around, with no weakness.

Oct. 15. The child was perfectly well.

(Temperature and pulse curves, Chart 1.)

Case 971.— E. S., a girl, aged 9 years. (Patient of Dr. A. B. Kuhl, Davenport Iowa.)

Sept. 19.—Headache, nausea and vomiting began 2 days previously and pain in the abdomen the day before. 4 p. m.—There was pain and stiffness in the back of the neck and the girl was drowsy. The knee jerks were diminished. The Kernig sign was present. The temperature was 102 F., the pulse 130. The thyroid was enlarged; the tonsils were large and 3 enlarged glands were noted outside either tonsil. There was no weakness. Spinal puncture was made, with spurting of fluid, and 15 c.c. were obtained. Cell count 8, globulin test + Ten c.c. of serum were given.

Sept. 20.—The temperature and pulse were normal and she felt well, with no weakness.

Sept. 21.—The girl appeared perfectly well and there was no weakness.

Oct. 15.—Recovery was complete without paralysis.

CASE 972.—M. J., a girl, aged 8 years; sister of Case 964. (Patient of Dr. A. Grassau, Princeton, Iowa.)

Sept. 20.—She complained of pain in the upper abdomen the day before. There was no fever in the morning but a temperature of 101 F. in the afternoon. She was extremely restless and had developed tremor of the muscles of the head and arms during the night. Stiffness of the neck and marked twitchings of the muscles were noted in the night. 10 a. m.—The tonsils were large and a large amount of peculiar pus was expressed from the left, and a small amount from the right. Two cervical glands were palpable on either side of the neck just outside of the tonsil. There was marked retraction and stiffness of the neck and back. Attempts to flex the head and the trunk caused severe pain. She was very tremulous and ataxic. The knee jerks were markedly exaggerated. Kernig on both sides. The pupils were dilated and the thyroid was enlarged and soft. A spinal puncture was made with spurting of turbid fluid; 10 c.c. were obtained. Cell count 800, globulin test ++. Ten c.c. of serum given. The muscle twitching was lessened immediately after the injection of the serum. 6 p. m.—The stiffness in the neck and the tremor had decreased.

Sept. 21.—The child looked brighter and the twitchings had disappeared. There was less retraction of the head and the neck and back were not so stiff. No weakness was evident. The right knee jerk was normal, the left slightly exaggerated. Kernig less marked. The temperature was 99.4 F., the pulse 130. A slight tremor of the muscles was noted under excitement while the serum was being injected. Ten c.c. of serum were given.

Sept. 22.—The girl looked brighter and no twitching of muscles was noted. The pupils were normal in size and the face was symmetrical. She walked without dragging the foot and without ataxia. There was no weakness. The knee jerks were normal. A spinal puncture was made; the fluid was under increased pressure and 2 c.c. were obtained. Cell count 500, globulin +. Six c.c. of serum were given.

Sept 23.—She appeared well, the stiffness of the neck and back and the twitchings of the muscles had disappeared. No weakness was observed.

Oct. 14.—Recovery was complete without paralysis.

(Temperature and pulse curves, Chart 1.)

CASE 975.—J. N., a boy, aged 5 years. (Patient of Dr. L. F. Sullivan, Donahue, Iowa.)

Sept. 21.—He had had an attack of follicular tonsillitis 2 weeks previously with a slight fever lasting for 2 days. He then seemed well until 4 days before the onset of the present illness, when he developed diarrhea, followed the next day by fever, severe headache, and vomiting. He was restless and nervous with a temperature the night before of 104.4 F., and a pulse of 130. He had been drowsy the preceding day and was extremely restless during the night, having had a number of attacks of marked twitchings of the muscles of the extremities. 4 : 30 p. m.—There was doubtful weakness of the left hand, but otherwise no paralysis. The tonsils were large, and palpable glands were found on either side. He appeared abnormally drowsy, could be aroused but fell asleep promptly. The head was retracted. Moderate Kernig. There was a definite stiffness of the neck. The right knee jerk was exaggerated, the left obtainable only on reinforcement. The temperature was 102.4. A spinal puncture was made

with spurting of fluid, and 10 c.c. were obtained. Cell count 83, globulin test +. Ten c.c. of serum were given.

Sept. 22. 10 a. m.—The knee jerks were normal. No weakness was evident. The temperature was 99.8 F. Seven and one-half c.c. of serum were given.

Sept. 23.—He appeared well, the temperature was normal, and there was no weakness.

Oct. 16.—The child was perfectly well. There was slight dragging of the right foot for 1 week after he got up.

CASE 979.—E. S., a girl, aged 4 years. (Patient of Dr. F. O. Burk, Davenport, Iowa.)

Sept. 24.—The patient awoke at 2 o'clock in the morning with severe generalized tremors, a high fever, vomiting and diarrhea. 11 a. m.—There was almost continuous tremor with sudden spasms of the muscles of the extremities, face, neck and eyes. The temperature was 103.8, the pulse extremely rapid. The girl lay in bed with head retracted and in a semicomatose condition from which she could scarcely be aroused. There were repeated involuntary urinations and defecations, the stools were offensive and contained a large amount of mucus. The body was extremely hot, the extremities were cold, and the skin was alternately pale and flushed. A moderate cyanosis was present. The left knee jerk was markedly exaggerated, the right absent. Bilateral Babinski and Kernig. The neck was stiff. The tonsils were large and large lymph glands were noted on the left side; none on the right. Spinal puncture was made and 4 c.c. of fluid were obtained. Cell count 19, globulin test +. 12:15 p. m.—Eight c.c. of serum were given. 3:30 p. m.—The condition was decidedly better and the child could be aroused. The facial expression was less anxious and she lay quiet. The temperature was 101. The right knee jerk was obtainable, the left normal. Kernig absent. The stiffness of the neck was less marked. She slept quietly with only occasional twitchings of the muscles of the forearms. She had not had sudden spasms of muscles since the injection of the serum. Ten c.c. of serum were given. 9 p. m.—The picture was entirely changed. The child was bright and rested quietly. The tremors and spasms had disappeared, the knee jerks were normal and Babinski and Kernig signs were absent. The temperature was 99 F., the pulse 112.

Sept. 25, 2 p. m.—The temperature was normal, the pulse 108. The child was rational, looked perfectly well and smiled. The pupils were equal, the tongue protruded in the median line and the reflexes were normal. There was slight rigidity of the neck. There was no weakness.

Oct. 15.—Recovery was complete without paralysis.

(Temperature and pulse curves, Chart 1.)

CASE 988.—C. L., a boy, aged 8 years. (Patient of Dr. F. C. Skinner, Le Claire, Iowa.)

Sept. 27.—The patient had gone to bed feeling well and awoke about 6 a. m. with headache, pain in the stomach and vomiting. He did not vomit food but material containing much mucus. The vomiting persisted and one loose bowel movement occurred. There was marked tremor over the whole body at 7 a. m. The temperature at 9 a. m. was 104., the pulse 160. 11:45 a. m.—He complained of headache, of stiffness of the neck, pain in the back of the neck, was apathetic and looked sick. There was tremor of the eyelids, the tongue was tremulous and there was tremor of the extremities. The throat was diffusely hyperemic, the tonsils were large and pus was expressed from the pole of the left. The follicles were empty. Two large glands were palpable on the left side just outside the tonsil; none on the right. The temperature was 104, the pulse 160.

The knee jerks were increased. Kernig absent. The neck was held rigid. A spinal puncture was made; the fluid was clear and under moderate pressure. Cell count 8, globulin test weakly positive. Ten c.c. of serum were given. 11:30 p. m.—He looked brighter. The temperature was 104, the pulse 120. Ten c.c. of serum were given.

Sept. 28.—The temperature was 100, the pulse 96. The stiffness of the neck had disappeared. He felt much better and there was no weakness.

Sept. 29, 9 p. m.—The temperature was 98.6, the pulse 74. The boy felt well and there was no weakness.

Oct. 15.—There was complete recovery without paralysis.

(Temperature and pulse curves, Chart 1.)

Case 996.—E. B., a girl, aged 6 years. (Patient of Dr. W. H. Rendleman, Davenport, Iowa.)

Sept. 28.—The patient went to bed apparently perfectly well the night before and got up in the morning with pain in the stomach. She vomited at noon and diarrhea began in the afternoon; the stools had a very foul odor. She was drowsy and sleepy all day. Could be aroused but went to sleep immediately. The temperature was 101.4 the pulse 130. She complained of headache in the morning and there were marked twitchings of the muscles at intervals, especially of the shoulder. 7 p. m.—The face was flushed, the head retracted and there were twitchings of the muscles. She had cramps in the stomach, followed by a bowel movement. The tonsils were large and diffusely red. There was considerable secretion in the throat. A lymph gland was noted on the left side. The left knee jerk was normal, the right absent. There was moderate distention of the abdomen. The temperature was 102.4, the pulse 144. A spinal puncture was made, with spurting of fluid, and 10 c.c. were obtained. Cell count 22, globulin test +. Ten c.c. of serum were given.

Sept. 29.—The temperature was 98.6, the pulse 110. The girl felt well and was up and dressed.

Oct. 2.—There had been severe cramps 2 days before, followed by 2 large bowel movements containing blood; none had occurred since. The reflexes, temperature and pulse were normal. There was no weakness.

Oct. 7.—There was complete recovery without paralysis.

(Temperature and pulse curves, Chart 1.)

RESULTS

Group 1.—*Patients showing no paralysis at the time of the serum treatment.* The 16 patients in this group recovered without paralysis (Table 1). One of these (Case 975), in which the serum treatment was begun on the fourth day, dragged the right foot for 1 week, but since the patient was not asked to walk on account of the severity of the symptoms, this may have been present at the time the serum was given.

The ages of the patients in this group ranged from 1-24 years, the average being 7 years. Eight were males and 8 females. The spinal fluid was under increased pressure in all; in two it was distinctly turbid, and in the others clear. The amount withdrawn ranged from 1.5-20

c.c., the average being 11 c.c. The cell count ranged from 8-800 per cubic millimeter, the average being 95. The globulin content was increased in all. The temperature was relatively high at the time of the first injection (Table 1). In 4 patients it was between 101 and 102; in 8 between 102 and 103; in 4 between 103 and 104. The pulse was very rapid in practically all.

The serum treatment was begun on the first day in 7 patients, on the second day in 2, on the third day in 5 and on the fourth day in 2. One dose only was given in 7 patients, two in 7, and three in 2. The amounts given ranged from 6-26 c.c., the average being 15 c.c. A prompt drop in the temperature and the pulse rate occurred in nearly every instance when invasion of the central nervous system, as manifested by the cell count in the spinal fluid, was not too great, quite irrespective of the previous duration of symptoms (Chart 1). The

TABLE 1

SUMMARY OF CASES SHOWING NO PARALYSIS AT THE TIME OF SERUM TREATMENT

Case No.	Sex	Age, Years	Condition of Patient	Spinal Fluid		
				Amount Withdrawn, C.c.	Cell Count	Globulin
936	M	9	Irritable, nervous retraction of head, exaggerated reflexes	15	60	+
944	M	11	Rigidty of neck and back. Left knee jerk diminished, right exaggerated	15	16	+
950	F	2	Drowsy, retraction of head, neck stiff, twitching of muscles, tremulous	20	133	++
953	M	3	Drowsy, rigidity of neck, bilateral Kernig, twitching of muscles	10	18	+
954	M	9	Rigidity of neck. right knee jerk exaggerated, left diminished, double Kernig	3	11	+
956	M	5	Double Kernig, tremulous, twitchings of muscles	10	44	+
962	F	24	Headache, pain in back, marked general weakness, rigidity of neck, knee jerk weak	20	23	++
964	F	4	Mental apathy, double Kernig and Babinski, hyperesthesia, tremulous, twitchings of muscles	10	211	+
966	M	3.5	Retraction of head, tremor of muscles, ataxia, right knee jerk diminished, left exaggerated	12	27	++
968	F	1	Retraction of head. rigidity of neck, knee jerks exaggerated, ataxia	1.5	40	+
971	F	9	Apathy, back and neck rigid. Double Kernig	15	8	+
972	F	8	Marked rigidity of neck and back. Tremulous and ataxic. Double Kernig	10	800	++
975	M	5	Rigidity of neck. Right knee jerk exaggerated, left weak	10	83	+
979	F	4	Marked generalized tremor of muscles. Retraction of head. Semi comatose. Left knee jerk exaggerated, right absent	4	19	+
988	M	8	Rigidity of neck and back. Tremor of muscles of eyelids and extremities	8	8	+
996	F	6	Retraction of head. Twitchings of muscles. Right knee jerk absent	10	22	+

drop in temperature occurred without an initial rise. The symptoms often disappeared in an astonishingly short time. Improvement began in some instances while the serum was being slowly injected or soon thereafter. A restless, sleepless, hyperesthetic child would often fall asleep soon after the administration of the serum. Twitchings and tremor of the muscles often became less or disappeared within a few hours after giving the serum. In 7 patients the temperature dropped to normal and the symptoms practically disappeared within 24 hours, in 7 they disappeared within 48 hours, and in 2 within 72 hours (Chart 1).

<div align="center">

Cases Showing Slight Paralysis at the Time of
Serum Treatment

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Case 939.—G. R., a boy, aged 7 years. (Patient of Dr. H. M. Decker, Davenport, Iowa.) The boy had had meningitis at 7 months, and three or four attacks of pneumonia. He had been well for 16 months, although not very strong.

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TABLE 1—*Continued*

Summary of Cases Showing No Paralysis at the Time of Serum Treatment

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Day of Dis- ease	Temper- ature at Time of Treat- ment	Total Amount of Serum Given, C.c.	Result
2	101	10	Temperature dropped by crisis. Prompt recovery without paralysis
3	104	12	Recovery without paralysis
3	101.5	6	Temperature disappeared by crisis. Prompt recovery without paralysis
1	101.5	10	Temperature dropped by crisis. Prompt recovery without paralysis
4	103	17	Temperature dropped by crisis. Prompt recovery without paralysis. Attack of appendicitis 4 days later
1	103	15	Temperature dropped by crisis. Complete recovery without paralysis in 24 hours
1	103.5	12.5	Temperature dropped by crisis. Prompt recovery without paralysis
3	103	26	Complete recovery without paralysis
3	103	17.5	Prompt recovery without paralysis
1	103	12	Prompt recovery without paralysis
3	102	10	Prompt disappearance of temperature. Recovery without paralysis
2	102.5	26	Recovery without paralysis
4	102.4	17.5	Critical drop in temperature. Slight dragging of right foot for 1 week. Complete recovery
1	103.8	18	Temperature dropped by crisis. Marked improvement in 3 hours. Complete recovery without paralysis
1	104	20	Temperature dropped by crisis. Complete recovery without paralysis in 24 hours
1	102.4	10	Temperature dropped by crisis. Complete recovery without paralysis in 24 hours

Aug. 29.—He was listless; played at intervals and was constipated.

Aug. 30.—The listlessness increased and he lay around. He was constipated and vomited in the evening.

Aug. 31.—Very listless, he slept most of the time and was still constipated. There was no apparent fever or pain.

Sept. 1.—He was in a semicomatose condition, was awakened with difficulty and could not be kept awake to answer questions. The temperature was 102, the pulse 100, the reflexes and strength were normal. There was moderate distention of the abdomen, the throat was faintly reddened and the tonsils were normal in size. There was no glandular enlargement, cough or coryza.

Sept. 2.—The temperature was 101.8, the pulse 90, otherwise the condition the same as the day before. 8 a. m.—Condition the same, semicomatose, the neck held rigid, and attempts to flex the head caused pain. The temperature was 102, the pulse 100. The speech was stuttering and he had hallucinations. 3 p. m.—Condition much the same. He complained of pain in the region of the right ear and the mastoid. The reflexes were normal, the strength was good, the throat was somewhat reddened. No pus could be expressed from the tonsils; one lymph gland outside the right tonsil was enlarged. The child was mentally confused and could scarcely be aroused. There was no weakness. 6 p. m.—The mental condition was growing worse, there was weakness of the right angle of the mouth. The temperature was 101, the pulse 100. A lumbar puncture was made and 0.3 c.c. of fluid obtained; a moderate amount escaped through the line of puncture after the needle was withdrawn. Cell count 78, globulin test +. Seven and one-half c.c. of serum were given.

Sept. 3, 7:30 a. m.—The temperature was 98, the pulse 72. The child was quiet and ate a bowl of oat-meal with relish. He was mentally clear but still somewhat sluggish. There was no extension of paralysis. 1 p. m.—He was mentally brighter.

Sept. 4, 7 a. m.—He was mentally normal and slept well; there was no extension of the paralysis. He had no pain anywhere and walked normally. The weakness of the right angle of the mouth was less marked.

Sept. 5.—There was slight weakness of the right corner of the mouth; otherwise he was normal.

Oct. 15.—The recovery was complete.

(Temperature and pulse curves, Chart 2.)

CASE 940.—B. B., a girl, aged 10 months. (Patient of Dr. F. Neufeld, Davenport, Iowa.)

Sept. 4.—The child was fussy and feverish the day before. There was fever, restlessness and twitching of the muscles of the hands and face in the night. During the forenoon the temperature was 105.2, the pulse 180. The bowels were flushed. By noon there were twitchings of the muscles of the face and arms bordering on slight convulsive attacks. 9:30 p. m.—The temperature was 104. There was twitching of the muscles of the face and fingers, the head was retracted, the eyes turned back, and attempts to flex the head caused the child to cry out. There was doubtful weakness of the muscles of the right angle of the mouth. The stools contained a large amount of greenish mucus. A spinal puncture was made, with spurting of fluid. Ten c.c. of slightly cloudy fluid were withdrawn. Cell count 155, globulin test +. Six c.c. of serum were given. 11:30 p. m.—The bowels were flushed with sodium bicarbonate and salt solution.

Sept. 5, 7 a. m.—The temperature was 101.5, the pulse 104. The child appeared brighter; had slept most of the night. There was still slight retraction of the head and stiffness of the neck. 11 a. m.—The temperature was 101, the pulse

125. The child slept quietly; there was no retraction of the head, no weakness anywhere. The stools were greenish and contained undigested milk curds. Five c.c. of serum were given.

Sept. 6.—The child appeared well in every respect. She laughed and kicked vigorously with both legs. The weakness of the right side of the mouth had disappeared.

Oct. 15.—Recovery was complete.

(Temperature and pulse curves, Chart 2.)

CASE 946.—F. C., a boy, aged 5 years. (Patient of Dr. F. Lambach, Davenport, Iowa.)

Sept. 9.—The boy complained of headache 2 days before and was feverish. The symptoms were thought to be due to enlarged tonsils, and arrangements were made for their removal. During the night a high fever developed.
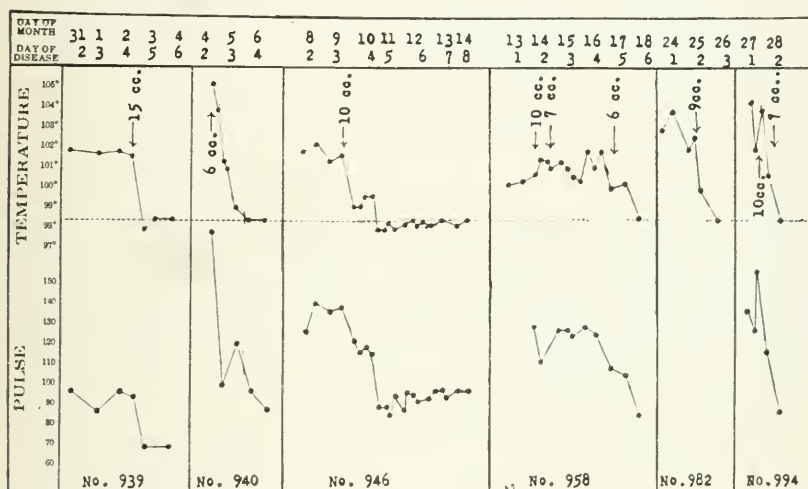
Chart 2. Temperature and pulse curves of patients showing slight paralysis at the time of serum treatment.

Sept. 10.—He vomited twice during the day and was unusually sleepy. The tonsils became more enlarged, and pus was expressed from the left. There were several enlarged lymph glands outside the left tonsil; none on the right side. There was marked tremor of the muscles of the jaw, noted especially on examining the throat. There were peculiar jerky movements of the muscles of the hands and about the eyes. The child walked normally. There was no evident weakness and no stiffness of the neck. The temperature was 102.5, the pulse 130. 10 p. m.—The child had been flighty and nervous during the day and very drowsy. He slept most of the day with the mouth open, the eyes partly closed, and the head retracted. He could be aroused but would fall back to semicomatose sleep immediately. The head was retracted and the neck stiff. There were Kernig and Babinski signs on both sides, the left knee jerk was absent and the right sluggish. There was marked ataxia in walking and the left foot dragged. There were peculiar jerky movements of the arms, and some difficulty in swallowing. He was unable to raise the eyelids normally, especially the left. The tem-

perature was 101.5. 10 : 30 p. m.—A spinal puncture was made; the fluid was under pressure and 15 c.c. were obtained. Cell count 18, globulin test + +. White blood count 10,100. The listlessness and inability to keep awake continued for one-half hour after spinal fluid was withdrawn. 11 p. m.—Ten c.c. of serum were given. 11 : 15 p. m.—The child had brightened up, took an interest in things and talked for fully half an hour. In puckering his mouth attempting to whistle and in showing his teeth it was noticed that the left side of the face was partially paralyzed.

Sept. 11.—He had slept quietly all night and appeared bright. The rigidity of the neck was less marked. Kernig and Babinski signs were absent on both sides. The knee jerk on the right side was normal; on the left side a number of vigorous kicks were obtained. The child walked without dragging the left foot. He opened his eyes normally but there was still slight weakness of the left side of the face when he attempted to whistle, but less marked than the night before. The temperature was 99.2. White blood count 11,800.

Sept. 12.—The temperature was 98, the pulse 90. He felt perfectly well; all weakness of the muscles, including those of the left side of the face, had disappeared.

Oct. 15.—Complete restoration of muscle function.

(Temperature and pulse curves, Chart 2.)

CASE 947.—H. L., a male, aged 20 years. (Patient of Dr. F. E. Rudolf, Davenport, Iowa.)

Sept. 10.—Two days previously he noticed a peculiar uncontrollable tremor of the hands, at times approaching spasms. He had no appetite, was nauseated, but did not vomit, and was constipated. He slept poorly the night before, was extremely nervous, and tossed about. He had repeated attacks of tremor and twitching of the muscles of various parts of the body. There had been severe headache the day before and tremor of the muscles. In the morning he noticed a peculiar thickness of the tongue and he was unable to swallow toast but could wash it down with liquids. 1 p. m.—While in the doctor's office he was very drowsy; said his tongue felt thick and tended to go to the roof of the mouth when swallowing. He was extremely restless and nervous; the muscles of various parts of the body, especially the right arm and leg, and the lips, twitched markedly at short intervals. He was ordered to go home to bed. 3 : 30 p. m.—While the history was being taken the patient dropped off repeatedly into partial sleep. He complained of headache and of feeling very nervous. There was twitching of the muscles of the right arm and leg and the lips at short intervals. The temperature was 99.8, the pulse 96. White blood count 8,700. The tonsils were large, and cheesy material was expressed from both. The muscles of the tongue were weak. There was slight rigidity of the neck and ataxia. The right knee jerk was exaggerated, the left normal. He was unable to swallow toast. 4 p. m.—A spinal puncture was made; the fluid was under pressure and 35 c.c. were withdrawn. Cell count 9, globulin test + +. 4 : 20 p. m.—No relief from the headache; twitching of the muscles continued. Twenty-two c.c. of serum were injected intravenously. While the injection was being made the patient stated with some surprise that his headache had disappeared and that he felt very much better. The twitchings of the muscles disappeared also before the injection of the serum was completed. 5 p. m.—The twitching of the muscles had not returned; the thickness of the tongue was less marked. The patient, much to his astonishment, was able to swallow toast.

Sept. 11, 9:30 a. m.—The headache and jerking of muscles had not returned. The thickness of the tongue had disappeared completely by 6 p. m. the night before. There was not the slightest difficulty in swallowing, the stiffness of the neck had disappeared, the temperature was normal, and the pulse 78. White blood count 9,000. The patient stood erect and walked without ataxia. There was no weakness, the knee jerks were equal and normal, the face was symmetrical. He begged for something to eat and insisted that he was perfectly well.

Oct. 15.—Urticaria 6 days after giving the serum. Recovery was complete.

This patient had had diphtheria antitoxin 13 years previously. He recovered without paralysis and with only slight serum disease.

Case 951.—L. R., a male, aged 23 years, employed as a truck driver. (Patient of Dr. D. G. Kreul, Davenport, Iowa.)

Sept. 12.—The patient had contracted a cold 5 days before. He sneezed, the nose was congested, and there was much discharge the first 2 days. At that time there was soreness of the throat and slight fever. He had had diarrhea 2 days previously. The present illness began the afternoon before, the patient felt achy and nervous; his head ached and he began to have attacks of twitching of the muscles in various parts of the body. An intense headache developed suddenly the next morning while he was on the way to work. Two hours later he vomited, was unable to walk, and had to lie down. The jerkings of the muscles became much worse. He was taken home and put to bed. 2 p. m.—He complained of severe headache, nausea, tightness in the chest, and dry cough. The tonsils were large and red; no attempt was made to express pus. There was a tender gland just outside the right tonsil, and several palpable glands on the left side. The patient lay in bed with a peculiar expression of tension on his face. He appeared nervous, and marked twitchings occurred at short intervals of muscles of various parts of the body, especially of the left arm. The right knee jerk was exaggerated, the left barely obtainable, and only on reinforcement. There was weakness in the legs, especially the left; the gait was ataxic. There was marked weakness in the extension of the left hand, none of the right. The temperature was 99.6. A spinal puncture was made with spurting of fluid and 30 c.c. were obtained. Cell count 18, globulin test +. No relief followed the spinal puncture. Twenty c.c. of serum were given.

Sept. 13.—The nurse said that he had had only 2 jerking spells after the injection of the serum. He had been restless during the first part of the night but had slept well and quietly during the latter part. In the morning he felt like getting up and going to work. He looked well. The knee jerks on both sides were normal. The cough persisted. The extension of the left wrist was powerful; the weakness of the left leg had disappeared; there was no weakness. The temperature was normal. Twelve c.c. of serum were given.

Sept. 14.—The patient appeared perfectly well.

Oct. 14.—Recovery was complete.

Case 952.—M. D., a girl, aged 15 months. (Patient of Dr. T. D. Starbuck, Davenport, Iowa.)

Sept. 12.—The child had been irritable and fussy, and had had diarrhea for two weeks. She had vomited at intervals the day before and during the morning. There was apparently no fever until the night before. At midnight severe convulsions occurred, and during the morning there were 9 convulsions which had to be controlled with ether. A drawn condition of the left side of the face was noted. A cough had existed for 2 days associated with râles in

the chest. She did not want to be handled. 3 : 45 p. m.—The temperature was 103. The muscles of the right hand twitched, and there was weakness of the muscles of the left side of the face and the left eyelid. The head was retracted, and attempts to flex it caused pain. The knee jerks were exaggerated. Babinski's sign was present. A spinal puncture was made; the pressure was increased and 10 c.c. of fluid obtained. Cell count 22, globulin test + +. Six c.c. of serum were given.

Sept. 13.—The child had had no convulsions since the injection of the serum. The highest temperature was 99.2. She appeared bright and the weakness of the left side of the face and the left eyelid was less marked.

Sept. 14.—She appeared perfectly well. Her temperature was normal and she walked normally. The weakness of the left side of the face and the left eyelid had disappeared.

Case 958.—T. D., a boy, aged 5 years. (Patient of Dr. E. F. Strohbehn, Davenport, Iowa.)

Sept. 14.—The child was perfectly well until 2 o'clock 2 nights before, when he became restless and feverish. He vomited repeatedly in the morning, the vomitus containing a large amount of mucus but no blood. There was no diarrhea. He complained of severe headache. He seemed very drowsy and talked constantly in his sleep during the night. He complained of pain in the small of the back, and in the morning showed twitchings of various muscles of the body, with severe pain in the back. There was no rigidity of the neck during night or morning. The temperature in the night was 100.6; in the morning it was 100.4. 11 : 30 a. m.—He was restless, and lay with head retracted. There was twitching of the muscles of the hands and of the legs. The face was flushed, the neck stiff and the child was unable to bring chin to chest. Flexion of the head caused pain in the neck and back. Kernig +. The knee jerks were exaggerated. The temperature was 101.4, the pulse 112. There was weakness in the extensors of the right leg; no other weaknesses could be detected. The tonsils were large, the throat hyperemic and moist, and there was a palpable lymph gland on either side of the neck just outside the tonsil. 11 : 45 a. m.—A spinal puncture was made; the fluid was under pressure and slightly turbid, and 10 c.c. were obtained. Cell count 141, globulin test + +. Ten c.c. of serum were given. Ten minutes after the injection was finished the boy said his headache had disappeared. 6 p. m.—The temperature was 101.4, the pulse 130. The back was rigid, the left knee jerk was normal, the right diminished. There was no increase in weakness of the right leg, and no weakness otherwise. 10 p. m.—The temperature was 101.2, the pulse 130. There were slight twitchings of the muscles of the mouth, the eyelids, and the arms, and less rigidity of the neck. The left knee jerk was exaggerated, the right diminished. Seven and one-half c.c. of serum were given.

Sept. 15, 1 : 40 p. m.—The child looked well. There was less twitching of the muscles about the mouth and eyes, the face was symmetrical and less flushed. The increase in the weakness of the right leg was moderate, and he was able to raise the leg in extended position.

Sept. 17, 12 m.—The temperature was 100.4. The weakness in the right leg had increased and he was unable to lift his foot from the bed in the extended position, but could move his toes. The extensors of the right foot were unimpaired. Six c.c. of serum were given.

Sept. 18.—The temperature was normal. There was a slight stiffness of the neck; the right knee jerk was absent. There was definite improvement in muscle power in the right leg and in the back and no weakness otherwise.

Oct. 15.—There was slight weakness in the right thigh and in the back which was decreasing rapidly.

(Temperature and pulse curves, Chart 2.)

Case 961..—H. B., a girl, aged 11 months. (Patient of Dr. H. M. Decker, Davenport, Iowa.)

Sept. 15.—The child had been well until 3 days before when she developed fever and began to vomit. There was no bowel disturbance and the fever continued. She had been abnormally drowsy since the onset. The temperature was 102 (the morning of Sept. 15) and for the first time it was noticed that the child was unable to hold her head up when sitting up. 3 p. m.—The tonsils were small, and no attempt was made to express pus. There were 2 small lymph glands on the right side of the neck just outside the tonsil. The left side of the face drooped. A marked weakness of the muscles of the neck, and undoubted difficulty in swallowing were noted. The voice was weak. A spinal puncture was made; the fluid was under pressure and 10 c.c. were obtained. Cell count 130, globulin test +. Five c.c. of serum were given.

Sept. 16.—The temperature was normal; the child appeared well. There was marked improvement in the muscles of the neck and the right side of the face.

Oct. 15.—Complete restoration of muscle function.

Case 969.—L. M., a girl, aged 2 years. (Patient of Dr. A. Grassau, Princeton, Iowa.)

Sept. 19.—The child had contracted a cold and had had a severe cough 4 days previously. There was a high fever the following night, apparently none the next night, and again a high fever the night of the 18th; there was looseness of the bowels and vomiting. 4:30 a. m.—The temperature was 104.5. The patient was tremulous and had a number of attacks in which the muscles of various parts of the body, particularly of the face, jerked. 5:30 a. m.—Severe generalized clonic and then tonic convulsions occurred which had to be controlled with chloroform and opiates. 10 a. m.—The temperature was 102. She had a severe cough, was extremely tremulous, with the knee jerks on the right side exaggerated and on the left side weak. Undoubted weakness in the left leg was noted. There was paralysis of the internal recti; both eyes turned out sharply. The head was retracted, the neck was very stiff, and the right side of the face was partially paralyzed. There was marked Kernig. Coarse mucous rales were detected in various parts of the chest. A spinal puncture was made, with spurting of clear fluid, and 8 c.c. were obtained. Cell count 17, globulin test +. Ten c.c. of serum were given. Shortly after the injection of serum the tremor of the muscles of the extremities disappeared, and the child slept naturally. 8 p. m.—She seemed better. The twitchings were less marked and there were no spasms. The temperature was 102.

Sept. 20, 9 a. m.—The patient was brighter, and active. There were still twitchings of the muscles but no spasms. She moved her arms and legs actively and walked without tremor. The temperature was 100. The rigidity of the neck and the strabismus had disappeared. A slight drawn condition of the right side of the face was still present but less marked than the day before. Five c.c. of serum were given.

Sept. 21.—The general condition was much better. Twitchings of muscles had not occurred following the injection of serum the day before. There was no weakness of the muscles of the extremities. The head was held erect; she sat up without difficulty. Coarse mucous rales in the chest, especially over the right side, could be detected. The temperature was 102.4.

Sept. 22.—The temperature was normal. There was no weakness.

Oct. 14.—Recovery was complete.

Case 970.—A. M., a boy, aged 17 months. (Patient of Dr. H. U. Braunlich, Davenport, Iowa.)

Sept. 19.—Two weeks previously the child had had diarrhea with mucus in the stools for a week. He was apparently well until 4 days before (Sept. 15), when he developed a high fever and extreme restlessness. The next day he vomited, and on the next was abnormally sleepy with twitchings of the muscles of the face and the extremities. Temperature 102. 2 p. m.—The tonsils were normal in size, but a small amount of pus was expressed from the right. There was an enlarged gland outside the right tonsil, but not on the left side. The throat was red, the muscles of the tongue weak, and the head retracted. He appeared markedly apathetic. His eyes were rolled back and he cried out at intervals as if in pain. There was marked weakness of the muscles of the neck, the right side of the face, and the tongue. He could move his extremities but was unable to stand. Knee jerks were absent. There was marked Kernig. The temperature was 100.6. A spinal puncture was made, the fluid was under pressure and 10 c.c. were withdrawn. Cell count 194, globulin test +. Ten c.c. of serum were given.

Sept. 20.—The temperature was normal, the child appeared brighter; there was no extension of the paralysis.

Sept. 21.—There was no extension of the paralysis and an undoubted improvement on the right side of the face.

Sept. 23.—Marked improvement was shown in the power of the muscles of the legs, neck, and right side of the face.

Oct. 15.—Complete recovery of muscle function.

Case 974.—H. A., a girl, aged 8 years. (Patient of Dr. J. P. Comegys, Rock Island, Illinois.)

Sept. 20.—The girl had been perfectly well until 2 days before when she suddenly became extremely ill, with persistent vomiting, marked diarrhea and high fever. She vomited or attempted to vomit almost constantly during the first night and had numerous extremely offensive bowel movements which contained a large amount of mucus. The diarrhea continued after the administration of castor oil, and many movements containing greenish mucus and pus-like material mixed with blood had occurred the day before. Pain developed in the back of the neck and she was very shaky. 6 p. m.—The child appeared to be sick. There was marked tenesmus and numerous bowel movements containing a large amount of mucus and bloody pus. The eyes were sunken, the lips and tongue red. The head was retracted and attempts to flex the head and the neck caused pain. Kernig sign present. The knee jerks were markedly exaggerated. Weakness of the muscles of the right side of the face was noted. The tonsils were normal in size; the throat hyperemic and covered with mucus. A spinal puncture was made, the fluid was under moderate pressure and 10 c.c. were obtained. Cell count 44, globulin test +. Ten c.c. of serum were given. Fifteen minutes after the injection the knee jerks became normal on repeated tests.

Sept. 21.—There was no extension of paralysis. The drawn condition of the right side of the face was barely noticeable. The bowel condition was unchanged.

Oct. 16.—Recovery was complete.

Case 981.—C. R., a girl, aged 8 months. (Patient of Dr. Neufeld, Davenport, Iowa.)

Sept. 25.—The infant was weak and poorly nourished. She had had diarrhea one week previously, and 56 hours previously had become ill and feverish with general irritability and restlessness. She had a number of spells in which she shook. She lay in bed, was extremely restless and the head was retracted. 10:30 a. m.—She was apathetic and lay with head retracted. She cried at attempts to flex the head. There was marked tremor of the hands, at times approaching convulsive spasms. The knee jerks were exaggerated. There seemed to be some weakness in the extensors of the hands. The temperature was 102.8. A spinal puncture was made, the fluid spurted and was definitely turbid; 2 c.c. were obtained. Cell count 475, globulin test + + +. Seven c.c. of serum were given. 5:30 p. m.—She looked brighter and had taken nourishment. The temperature was 102. She had been much quieter, more contented, and had played most of the afternoon. 7:30 p. m.—The temperature was 100. She appeared to be feeling better. 9:30 p. m.—Sleeping normally without twitchings.

Sept. 26, 10:15 a. m.—The temperature was 100. She looked bright and playful and smiled. Her face was symmetrical; the reflexes were normal, and only slight rigidity of the neck was noted. No weakness. Five c.c. of serum were given.

Sept. 27.—Temperature and pulse normal. No weakness. The child appeared well.

Oct. 15.—Recovery was complete.

Case 977.—G. S., a girl, aged 2 years. (Patient of Dr. F. C. Skinner, Le Claire, Iowa.)

Sept. 23.—The child had had coryza and sore throat 5 days previously. One sister, a hired man, and the father had had similar attacks. She vomited 2 nights before. The temperature was 102. She became drowsy the following day with severe spasm and twitchings of muscles at midnight. 12:30 p. m.— There was slight rigidity of the neck. The left knee jerk was exaggerated, the right diminished and the right foot dragged slightly. The right eyelid drooped, and a weakness of the muscles of the right side of the face was apparent on crying. The throat was moist. The tonsils were large and there was an enlarged lymph gland on either side, just outside the tonsil. The temperature was 100.4, the pulse 112. A spinal puncture was made, with spurting of fluid, and 10 c.c. were obtained. Cell count 83, globulin test +. Six c.c. of serum were given.

Sept. 24.—The temperature was normal, the pulse 80. The drawn condition of the face and the weakness of the right foot had disappeared.

Oct. 15.—Recovery was complete.

Case 982.—H. R., a boy, aged 3 years. (Patient of Dr. James Dunn, Davenport, Iowa.)

Sept. 25.—Two nights previously the child had vomited repeatedly. Twitchings of the muscles had occurred the following day and in the night he had diarrhea with stools of foul odor and containing a large amount of mucus. He had a severe convulsion in the night lasting 20 minutes, and in the morning was stuporous. The temperature was 102. There were frequent movements of the bowels, containing much greenish mucus, but no blood. 11:45 a. m. —The child was extremely apathetic and the head was retracted. He could be aroused but would fall asleep immediately. The muscles twitched. The tonsils were large and there was much mucus in the throat. Pus was expressed from the left tonsil, but none from the right. There were a number of enlarged glands on the left side adjacent to the tonsil, but none on the right. The

knee jerk on the left side was increased, on the right side, diminished. There was decided weakness of the muscles of the right side of the face. A spinal puncture was made, the fluid was under pressure, and 12 c.c. were obtained. Cell count 25, globulin test +. Nine c.c. of serum were given. 6 p. m.—The temperature was 100. The child had rested quietly during the afternoon, he seemed brighter and talked. There was no change in the weakness of the right side of the face, and no weakness otherwise.

Sept. 26, 7:30 p. m.—He appeared well and bright, and was hungry. The looseness of the bowels continued. The temperature was 98.6. There were no twitchings and the knee jerks were normal.

Sept. 28.—The weakness of the right side of the face was absent; there was no other weakness. The bowel movements continued to contain a small amount of mucus. The temperature and pulse were normal.

Oct. 15.—Recovery was complete.

(Temperature and pulse curves, Chart 2.)

Case 986.—M. P., a girl, aged 5 months. (Patient of Dr. J. W. Shields, Moline, Illinois.)

Sept. 26.—The child had had a discharge from the nose 5 days previously, but otherwise seemed well until midnight when she had developed a high fever. The temperature at 6 a. m. was 102.2; at 8 a. m. 103.2. She vomited during the afternoon and was unusually drowsy. 5:30 p. m.—The tonsils were normal, the throat hyperemic and moist. The head was retracted, the neck stiff. Kernig sign present. The knee jerks were exaggerated and there was a doubtful weakness of the right arm. The right eye turned in at intervals. A spinal puncture was attempted, but no fluid was obtained. Five c.c. of serum were given.

Sept. 27.—The temperature was normal. The slight weakness in the right arm and the right eye had disappeared. The child was apparently well in every respect.

Oct. 15.—Recovery was complete.

Case 994.—L. A., a girl, aged 4 years. (Patient of Dr. A. E. Williams, Rock Island, Illinois.)

Sept. 27.—The child had apparently been perfectly well until noon, when she went to her mother asking to be held. She acted rather strangely, was extremely nervous and complained of pain in her throat. During the afternoon she complained of pain in her left foot. 2 p. m.—The temperature was 104.4, the pulse 140-160. 5 p. m.—She vomited, was very drowsy and apathetic, but restless and extremely ill. 6 p. m.—The temperature was 102, the pulse 138. She was extremely apathetic and could scarcely be aroused. The head was retracted, the eyes rolled back and there was a tremor of the muscles over the entire body. 9:30 p. m.—A generalized convulsion occurred with involuntary urination and defecation. She was alternately flushed and pale. 10 p. m. —The temperature was 104, the pulse 160. She was cyanotic, and the twitchings of the muscles of the face, hands and legs were severe. She became comatose and could not be aroused. There was weakness of the muscles of the right side of the face. The knee jerks were exaggerated, the head retracted and the neck stiff. Kernig and Babinski signs were marked. Spinal puncture was made with spurting of the fluid, and 10 c.c. were obtained. Cell count 19, globulin test +. Ten c.c. of serum were given. The twitchings disappeared during the injection of serum.

Sept. 28, 7 a. m.—She slept quietly for 2 hours after the serum was given, but grew restless again and had a second convulsion at 3 o'clock. The tremors

returned, lasted for an hour and then gradually disappeared. The temperature was 100.8, the pulse 120. The twitching of the hands was slight. She looked brighter. The weakness of the right side of the face had disappeared and there was no other weakness. 1 p. m.—Her color was good, she was active and begged for something to eat. She looked well. The knee jerks were normal. Kernig and Babinski signs were absent. There were no twitchings of any of the muscles. The temperature was 98.6, the pulse 88. There was no weakness. Seven and one-half c.c. of serum were given.

Sept. 29.—The child was perfectly well.

Oct. 15.—Urticaria 3 days after the serum treatment, and a slight edema of the eyelids 10 days later. Recovery was complete.

(Temperature and pulse curves, Chart 2.)

Case 1000.—R. P., a boy, aged 3 years. (Patient of Dr. E. W. Bittner, Wheatland, Iowa.)

Sept. 28.—He complained of pain in his stomach. His head had ached 4 days before and since then he had been feverish. There was a marked twitching of the muscles when he was asleep. He was unusually drowsy and lay with head retracted. The temperature was 101, the pulse rapid. Paralysis of the left side of the face began the day before. 10 p. m.—The paralysis of the left side of the face was almost complete and the weakness of the muscles of the neck was marked. His head would fall backward in bringing him to a sitting position. Strabismus was marked, and there was alternate flushing and pallor of the skin. He was extremely fretful and irritable. Ataxia was marked and the knee jerks were exaggerated. He was unable to shut the left eye. Spinal puncture was made; the fluid was under pressure and distinctly turbid, and 5 c.c. were obtained. Cell count 955, globulin test + +. Ten c.c. of serum were given.

Sept. 30, 4 p. m.—The parents stated that the boy became quieter soon after the injection of serum, and rested quietly during the night. He appeared brighter in the morning. The muscles of the right side of the face were stronger, which was especially noticeable when he laughed and cried. The temperature was normal, the pulse 85. The muscles of the neck were decidedly stronger. He could hold his head almost normally when brought to a sitting position, and he walked without ataxia. There was no apparent weakness of the arms or legs. He could close his left eye completely. Eight c.c. of serum were given.

Oct. 15.—Complete recovery except slight weakness of the left side of the face noticeable only on crying and laughing. Rapidly improving.

RESULTS

Group 2.—*Patients showing slight paralysis at the time of the serum treatment.* In 16 of the 17 patients in this group the paralysis was arrested, the fever and symptoms disappeared promptly and the restoration of function of the paralyzed or weakened muscles occurred rapidly, particularly in Cases 947, 951, and 994. The 16 patients were well with complete restoration of muscle-function in from 1-7 days after the serum was given. One patient (Case 958) who had severe infection of the tonsils with enlarged paratonsillar glands, showed a

slight extension of weakness in the right thigh, but 6 weeks later restoration of muscle function was nearly complete. The age of the patients in this group ranged from 5 months to 23 years, the average being 5 years (Table 2). Eight were males and 9 were females. The spinal fluid was under increased pressure in all the cases in which the puncture was successful. In one (Case 986) the dura was not punctured. In three the fluid was distinctly turbid, and in the others it was clear. The amount withdrawn ranged from 0.3 c.c. to 35 c.c.,

TABLE 2

Summary of Cases Showing Slight Paralysis at the Time of Serum Treatment

Case No.	Sex	Age, Years	Condition of Patient	Spinal Fluid		
				Amount Withdrawn, C.c.	Cell Count	Globulin
939	M	7	Stuporous, rigidity of neck, stuttering speech and hallucinations, weakness of right side of face	0.3	78	+
940	F	10/12	Twitching of muscles of face and fingers, weakness of right angle of mouth	10	155	+
946	M	5	Retraction of head, rigidity of neck, Kernig and Babinski both sides, left knee jerk absent, ataxia, weakness of left foot, eyelids, and left side of face	15	18	++
947	M	20	Twitching of muscles, weakness of muscles of deglutition and tongue, inability to swallow solids, headache	35	9	++
951	M	23	Headache, muscular twitching, right knee jerk exaggerated, left diminished, ataxia, weakness of left leg and arm	30	18	+
952	F	1 3/12	Twitching of muscles, rigidity of neck, weakness of muscles of left side of face	10	22	++
958	M	5	Rigidity of neck, double Kernig, muscular twitchings, weakness of extensors of right leg	10	141	++
961	F	11/12	Weakness of left side of face and muscles of neck. Slight difficulty in swallowing	10	130	+
969	F	2	Rigidity of neck, double Kernig. Tremulous, right knee jerk exaggerated, left very weak, weakness of right side of face, left leg and internal recti	8	17	+
970	M	1 5/12	Retraction of head. Marked weakness of muscles of neck, right side of face and tongue. Knee jerks absent	10	194	+
974	F	8	Weakness of muscles of right side of face. Knee jerks exaggerated. Rigidity of neck. Marked colitis	10	44	+
977	F	2	Knee jerks unequal. Weakness of right foot, right eyelid and right side of face	10	83	+
981	F	8/12	Retraction of head. Tremors. Knee jerks exaggerated. Weakness in extensors of hands	2	475	+++
982	M	3	Retraction of head. Weakness of muscles of right side of face. Inequality of knee jerks	12	25	+
986	F	5/12	Rigidity of neck. Double Kernig. Doubtful weakness of right arm			
994	F	4	Comatose. Severe twitching of muscles. Rigidity of neck. Double Kernig and Babinski. Knee jerks exaggerated. Partial paralysis right side of the face	10	19	+
1000	M	3	Almost complete paralysis left side of face. Marked weakness of muscles of neck. Strabismus. Marked ataxia	5	955	++

the average being 12 c.c. The cell count ranged from 9-955 cells per cubic millimeter, the average count being 149. The globulin test was positive in all. The temperature at the time of the first injection was between 98 and 102 in 12 of the cases, between 102 and 103 in 3, and 104 in 2. The pulse was unusually rapid as in the patients in Group 1.

The serum treatment was begun on the first day in 5 cases, on the second day in 6, on the third day in 2, and on the fourth day in 4. In 10 cases only one injection was given, in 6 two injections, and in 1

TABLE 2.—*Continued*

SUMMARY OF CASES SHOWING SLIGHT PARALYSIS AT THE TIME OF SERUM TREATMENT

Day of Disease	Temperature at Time of Treatment	Total Amount of Serum Given, C.c.	Result
4	101	7.5	Temperature dropped promptly, mental condition improved, weakness of face disappeared entirely
1	104	11	Temperature dropped abruptly. Prompt and complete recovery
2	101.5	10	Prompt disappearance of temperature. No extension of paralysis. Rapid and complete recovery
2	99.8	22	Headache and muscular twitchings disappeared during serum injection. Ability to swallow returned within 1 hour. Complete recovery in 24 hours
1	99.6	32	Prompt and complete recovery. No extension of paralysis
1	103	6	No extension of paralysis. Temperature dropped by crisis. Complete recovery
2	101.4	23.5	Distinct extension of paralysis of right leg. Ultimate complete recovery
3	102	5	Temperature disappeared promptly. No extension of paralysis. Complete recovery
4	102	15	No extension of paralysis. Rapid and complete recovery
4	100.6	10	No extension of paralysis. Marked early improvement. Complete recovery
2	98.6	10	No extension of paralysis. Complete recovery
2	100.4	6	No extension of paralysis. Complete recovery
3	102.8	12	No extension of paralysis. Complete recovery
2	102	9	Rapid disappearance of temperature. No extension of paralysis. Complete recovery
1	102.2	5	Prompt recovery
1	104	17.5	No extension of paralysis. Complete recovery within 24 hours
4	101	18	Prompt improvement following both serum injections. Almost complete recovery. Slight weakness of left side of face

three injections. The amount given ranged from 5-32 c.c., the average being 13 c.c. A prompt drop in temperature and pulse rate occurred in nearly all instances. The improvement in this group was quite as striking as that in Group 1, and could be measured more accurately by noting the improvement in the function of the weakened or paralyzed muscles. The drop in the temperature and pulse rate in this group, as in Group 1, occurred without an initial rise (Chart 2). The symptoms in some patients, as of those in Group 1, began to disappear while the serum was being slowly injected, or soon thereafter (Cases 947, 951, 994, and 1000) and in at least one patient (Case 994) unmistakable symptoms of a rapidly progressing bulbar type of the disease disappeared promptly after the injection of serum, recovery being practically complete within 24 hours.

CASES SHOWING ADVANCED PARALYSIS AT THE TIME OF SERUM TREATMENT

CASE 931.—H. M., a boy, aged 6 years. (Patient of Dr. D. G. Kreul, Davenport, Iowa.)

Aug. 29.—The patient was perfectly well until 8 days before, when for several days he had a slight fever and did not eat well. He then appeared well until 5 days later, when he complained of pain in the back of the neck and of feeling sick all over. He felt hot and vomited, was restless, tossed about and talked continuously in his sleep. There was pain and stiffness of the neck. The pain in the neck was aggravated on attempting to flex the head. The pulse was rapid and the temperature 100.5. The following day his condition was much the same but the temperature was 101.5. There was no evident paralysis. On the morning when first seen the pain and stiffness in the neck had disappeared. He awakened his father at 5 o'clock telling him that he could not move his right arm; his temperature was 101.6. 10 p. m.—There was almost complete flaccid paralysis of the right shoulder, extension of the right hand was weak, the grasping power quite well preserved. There was definite weakness in the right leg, but he could kick a hand with the leg held in extended position. The knee jerk on the right side was absent, on the left diminished. There was tremor of the muscles of the jaw and complaint of pain in the right ankle.

Aug. 30, 2 p. m.—Pain in the right ankle and leg was still present. The patient was restless and the paralysis had extended. Knee jerks on both sides were absent and the child was unable to lift the right leg in extended position. The left eyelid drooped, there was tremor of the muscles of the jaw and lips and marked weakness of the muscles of the neck. The temperature was 101.8, the pulse 140. A spinal puncture was made, the fluid was under pressure and 15 c.c. were withdrawn. Cell count, 135, globulin test +. Four c.c. of immune serum were injected intravenously. No untoward symptoms followed the injection. 9 p. m.—The child rested and slept quietly for 2 hours after the injection of serum, for the first time since the onset of the illness. The pain in the right leg had disappeared. Eight c.c. of serum were injected.

Aug. 31, 8:30 a. m.—The parents stated that the boy slept quietly without awakening until 4 a. m. He looked bright and appeared rested. The tremor

of the muscles of the jaw, and the drooping of the left eyelid had disappeared. There was no extension of paralysis in the right shoulder and he could again lift the right leg in extended position. The temperature was normal. 8:30 p. m.—Undoubted improvement had occurred in the right arm. He could reach for things on the opposite side of his body. He kicked a hand 1½ ft. above the bed with the right leg in extended position. The knee jerk on the left side had returned. Eight c.c. of serum were injected.

Sept. 2.—There was marked improvement in muscle power in the right arm and leg, and the muscles of the neck were stronger. He appeared well.

Sept. 27.—The boy was up and around and walked without dragging the right foot. There was still slight weakness in the muscles of the back. He was able to move the right arm in every direction, but could not yet hold the arm in a horizontal position.

Oct. 15.—There was almost complete restoration of muscle function.

(Temperature and pulse curves, Chart 3.)

CASE 932.—W. D., a boy, aged 3¾ years. (Patient of Dr. W. Matthey, Davenport, Iowa.)

Aug. 30.—There had been fever, vomiting, and diarrhea 4 days before. The child became very tremulous and nervous and this condition was soon followed by drowsiness and listlessness. A slight weakness of the left leg was noted 3 days later. The boy dragged the left foot, was cross and complained of pain in the left leg. 3 p. m.—There was evident weakness in both legs, but more marked in the left; he was unable to walk but could still lift the left leg in extended position. The knee jerk was absent on the left side and barely obtainable on the right. There was rigidity of the neck and attempts at flexion caused pain. 5 p. m.—The weakness in the legs had increased markedly. He was now entirely unable to lift the left leg from the bed or to flex the thigh. The temperature was 101.5, the pulse very rapid. A spinal puncture was made, The fluid was under pressure and 12 c.c. were obtained. Cell count 94, globulin test +. Four c.c. of serum were given; no untoward symptoms followed the injection.

Aug. 31, 1 p. m.—The general condition had improved. He was no longer drowsy or listless but appeared bright and had slept better than on previous nights. The paralysis had not extended. The knee jerk on the left side was still absent while that on the right was more vigorous. The temperature was normal. Seven and one-half c.c. of serum were given.

Sept. 1, 12 m.—A spinal puncture was made, the fluid was not under pressure, and 1 c.c. of clear fluid was obtained. The general condition was good. There was undoubted increase in strength in both legs. He complained of pain in the left leg. Six c.c. of serum were given. Improvement in muscle function was rapid. The pain and the tendency to draw up the leg continued for some time. Three weeks later the boy could ride a tricycle but was not yet quite able to bear his weight on the left leg.

CASE 933.—L. C., a girl, aged 8 years. (Patient of Dr. P. A. Bendixon and Dr. Wm. H. Rendleman, Davenport, Iowa.)

Aug. 31.—The tonsils and adenoids were removed 3 years previously and there had been no trouble with the throat since that time. The girl had an attack of indigestion 1 month previously but recovered completely following the administration of laxatives. She had remained well until a week before, since which time she had felt ill. Four days before she complained of having pain and a lump in her throat. Three days before she began to vomit, had fever, a temperature of from 101 to 102, and the vomiting continued. She

Case No.	Sex	Age, Years	Condition of Patient	Spinal Fluid		
				Amount Withdrawn, C.c.	Cell Count	Globulin
931	M	6	Almost complete paralysis of right arm and right leg, marked weakness of muscles of neck	15	135	+
932	M	3 9/12	Almost complete paralysis of left thigh. Paralysis rapidly progressing	12	94	+
933	F	8	Inability to swallow or speak, marked weakness of muscles of neck, beginning paralysis of respiratory muscles, persistent vomiting	30	165	+ +
934	M	2	Marked weakness of muscles of neck, paralysis of right side of face, paralysis extending	15	60	+
935	F	1 6/12	Paralysis of lower extremities, restlessness....	15	0	0
937	M	4	Marked cyanosis, pulse barely obtainable, complete paralysis of thorax and diaphragm, kept alive by artificial respiration	10	185	+ + +
938	M	12	Paralysis of arms, muscles of neck and thorax, inability to swallow, marked cyanosis, edema of lungs, extremities cold, pulse barely perceptible	20	320	+ + +
942	M	11/12	Semicomatose, rigidity of neck and left leg and arm, retraction of head. Weakness of left side of face, left index finger and left thumb	12	144	+
943	M	2	Extremely drowsy, marked weakness of left leg, ataxic and tremulous. Cyanotic, paralysis of left side of face, paralysis of upper part of chest	15	120	+
945	F	14	Progressing ascending paralysis, almost complete paralysis of legs and arms, and muscles of eyelids	15	130	+ +
948	F	11	Retraction of head, rigidity of neck, marked weakness of right side of face and eyelids, photophobia, muscles of face tremulous, marked weakness of left knee jerk, drowsy	20	316	+ +
949	F	17	Typical ascending paralysis, complete paralysis of legs, abdominal muscles and diaphragm, partial paralysis of thorax, arms, and muscles of deglutition, air hunger	20		
955	M	1 1/12	Paralysis of muscles of lower extremities, marked weakness of back, neck, and left arm, hyperesthetic and fretful	20	133	+ +
957	F	9/12	Marked weakness of muscles of left leg, back, neck and external rectus, and right eye	10	30	+ +
959	F	1	Comatose, cyanotic, mucous rattle in throat, inability to swallow, complete paralysis of lower extremities and upper thorax, twitching of eyeballs, extremities cold	10	58	+ +
960	F	16	Cyanosis. Persistent vomiting. Inability to swallow. Paralysis of external recti. Weakness of muscles of left arm and thorax	15	344	+ + +
963	M	3	Marked weakness of muscles of arms, legs, neck and pharynx, complete paralysis of external recti, twitching of eyeballs, difficulty in swallowing, paralysis rapidly progressing	3	277	+ + +
967	F	7	Comatose, combined flaccid and spastic paralysis of lower extremities. Kernig and Babinski signs, stiffness and retraction of head	10	50	+ +
978	F	3 6/12	Almost complete flaccid paralysis of right arm and left leg, weakness of muscles of neck	10	83	+ +
980	M	1 3/12	Stuporous, inability to swallow, complete paralysis of thorax and left side of diaphragm, marked weakness of muscles of neck, arms and left leg	10	347	+ + +
983	F	9/12	Paralysis of right arm and leg, retraction of head, eyes turned sharply to left, knee jerk of right side absent, left side exaggerated	12		
985	M	3 9/12	Marked weakness of right leg and thigh.......	10	222	+ + +
995	F	11	Ascending paralysis, complete paralysis of left leg, marked weakness of right leg and both arms	15	97	+ +
1008	F	11/12	Almost complete paralysis of right side of face and right leg, complete paralysis of right arm, weakness of muscles of back	2	8	+ +
1024	M	2	Marked weakness of right leg and arm and muscles of back, irritable, cross. Severe pain in legs	5	16	+ +

TABLE 3.—*Continued*

SUMMARY OF CASES SHOWING ADVANCED PARALYSIS AT THE TIME OF SERUM TREATMENT

Day of Disease	Temperature at Time of Treatment	Total Amount of Serum Given, C.c.	Result
3	101.8	20	Prompt drop in temperature. Paralysis arrested. Rapid recovery of muscle function
4	101.5	17.5	Prompt drop in temperature. Paralysis arrested. Early and marked improvement. Complete recovery assured
4	101.4	25	Paralysis arrested. Vomiting and temperature disappeared promptly. Rapid restoration of muscle function
3	98.6	6	Paralysis arrested. Almost complete recovery
14	98.6	5	Restlessness disappeared. No apparent effect on paralysis. Improvement slow
1		16	No apparent effect. Death from respiratory failure
3	105	17	Temporary improvement. Death from respiratory failure
8	100	25	Temporary improvement following 3 injections of serum. Death, apparently from exhaustion
1	103	16	Temporary improvement. Death from respiratory failure
4	101.5	24	Paralysis arrested, marked early improvement but marked weakness of legs, improvement continuing markedly 5 weeks later
2	102	36	Temporary improvement following first injection of serum. None following subsequent injection. Death from respiratory failure
4	101	35	Definite improvement following first injection. No apparent effect following subsequent injections. Died from respiratory failure
3	99.8	6	Temperature dropped to normal. Less restlessness. No extension of paralysis. Marked improvement. Will probably recover completely
5		6	No extension of paralysis. Marked improvement soon after giving serum
4	104	6	Temporary improvement. Died of respiratory failure
2	103	12.5	Temporary improvement. Died of respiratory failure
3	103.5	22	Paralysis arrested. Marked early improvement in muscle function. Complete recovery assured
14		10	No apparent effect. Died
4	99	10	Temperature and pulse rate became normal within 24 hours. No extension of paralysis. Improvement slow
3	100.6	12	Temporary improvement. Died, apparently of terminal bronchopneumonia
2	102.8	6.5	No extension of paralysis. Prompt improvement. Temperature normal within 24 hours
4	100	10	No extension of paralysis. Gradual improvement in muscle function
2	100	45	Paralysis arrested. Prompt improvement. Almost complete restoration of muscle function except in left leg 3 weeks later
4	99.6	17.5	Paralysis arrested. Prompt improvement at first, then gradual improvement. Complete recovery assured
14		12	Decided improvement in general condition. Pains disappeared. Decided gain in muscle function within 36 hours.

was unable to keep anything on her stomach. There was retraction of the head and pain in the neck and throat; she was extremely restless, nervous, irritable, and trembly at times. The day before she began to have trouble in swallowing and choked at every attempt to swallow.

Aug. 31, 2 p. m.—She had repeated choking spells in which she became cyanotic and death appeared imminent. She was unable to speak, her face was much distorted, she was utterly unable to swallow, and there were almost constant attempts at vomiting. The muscles of the pharynx and the tongue were very weak, the throat was diffusely hyperemic and thickly covered with mucus. There was a moderate amount of infected lymphoid tissue in the region of the left tonsil. The thyroid was enlarged. There was marked weakness of the muscles of the neck and tremor of the muscles of the forearm. The pupils were dilated. There was marked pallor. The temperature was 101.4, the pulse 160, and the respiration shallow. The expansion of the chest was diminished in its upper portion; the respirations were chiefly diaphragmatic and there was moderate cyanosis. A spinal puncture was made, the fluid was under marked pressure and 30 c.c. were withdrawn. Cell count 165, globulin test + +. Ten c.c. of serum were given. 9:30 p. m.—The girl had rested quietly most of the time since the injection of the serum. She had slept at intervals, the pulse was less rapid and of better quality. She could swallow better and the cyanosis was absent. Five c.c. of serum were given.

Sept. 1, 10 p. m.—There was a marked change in the condition of the patient. Cyanosis had disappeared. Expansion of the chest was normal. She could speak. The weakness of the muscles of the neck was less marked but there was still difficulty in swallowing. Vomiting had entirely disappeared. Fluids and nutrients were given per rectum. Ten c.c. of serum were given.

Sept. 2.—The temperature was normal, and there was marked change for the better in every respect. She could swallow small amounts of liquid with the head held to the left side. There was pain in the back of the neck. 9:30 p. m. —The pain in the back of the neck had disappeared, but there was some difficulty in swallowing for a week. There was general improvement from the time of the first injection of serum. Five weeks later there was no evidence of paralysis anywhere except a slightly drawn condition of one side of the face, and no difficulty in swallowing meat. The girl was perfectly well.

(Temperature and pulse curves, Chart 3.)

Case 934.—A. H., a boy, aged 2 years. (Patient of Dr. L. F. Newburn, McCausland, Iowa, and Dr. J. T. Haller, Davenport, Iowa.)

Aug. 31.—The patient was perfectly well until 3 days before, when he became drowsy and prostated; he vomited, had diarrhea and was feverish. There was retraction of the head. The temperature was 102. There was paralysis on the right side of the face 2 days later, but no other weakness was noted. 2 p. m.—There was marked weakness of the muscles of the neck; he was unable to hold his head erect. The right side of the face was completely paralyzed, he could not close the right eye, and the paralysis was rapidly extending. The temperature was normal, the throat and tonsils reddened, and a large amount of pus was expressed from the pole of the left tonsil, but none from the right. An enlarged lymph gland was noted outside the tonsil on the left side, but none on the right. The knee jerks were normal. A spinal puncture was made, the fluid was under pressure, and 15 c.c. were withdrawn. Cell count 60, globulin test +. Six c.c. of serum were given.

Sept. 1.—The temperature was normal. The child appeared bri 'er. There was no extension of paralysis.

Sept. 12.—There was complete recovery except slight weakness of the right eyelid.

This patient was in the country. A neighbor's child had recently died of "intestinal disturbance," with pain and rigidity of the neck. There had been no contact until 3 days before the onset of the symptoms in this child.

CASE 935.—C. S., a girl, aged 18 months. (Patient of Dr. D. G. Kreul, Davenport, Iowa.)

Aug. 31.—Typical onset 2 weeks previously with restlessness, vomiting, fever, hyperesthesia, pain in the back of the neck, retraction of the head, followed by almost complete paralysis of the lower extremities. Restlessness was still present, although the temperature had been normal for 10 days. The patient continued to have crying spells for an hour at a time and there was no improvement in the paralysis. Because of this it was thought that the serum might still do some good. 9 p. m.—A spinal puncture was made, the fluid was under
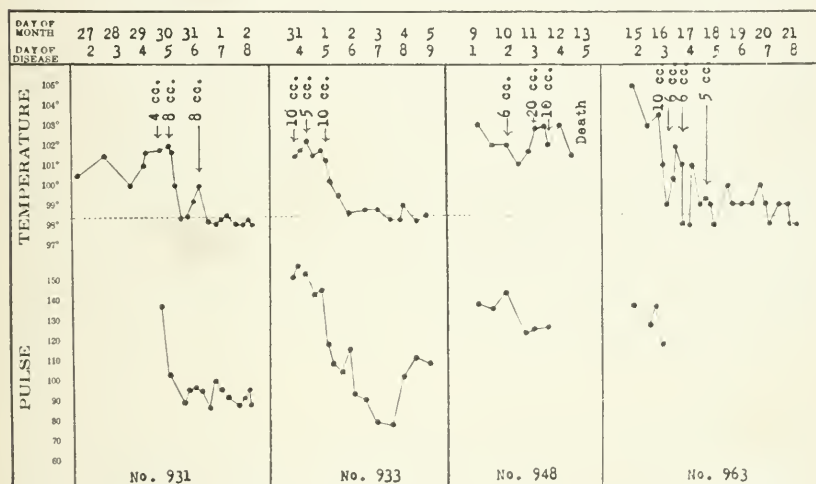
Chart 3. Temperature and pulse curves of patients showing advanced paralysis at the time of serum treatment.

slight pressure, and 15 c.c. were withdrawn. Cell count and globulin test negative. Five c.c. of serum were given.

Sept. 1.—There was no apparent change except that the baby rested better. The crying spells and the restlessness had disappeared.

Oct. 15.—Only moderate improvement in muscle function; marked weakness in legs.

CASE 937.—L. B., a boy, aged 4 years. (Patient of Dr. F. Neufeld, Davenport, Iowa.)

Sept. 1, 2 p. m.—There had been vomiting, diarrhea, headache, retraction of the head, marked tremor and high fever since the day before. On our arrival the patient was dying from respiratory failure. He was gasping for breath, there was marked cyanosis, the pulse was barely obtainable, and the extremities were cold. The thorax was immobile and there was very faint contraction of the diaphragm. He was kept alive by artificial respiration for a number of

hours. A spinal puncture was made, the fluid was under pressure, and 10 c.c. were obtained. Cell count 185, globulin test + + +. Two and one-half c.c. of serum were injected intraspinously and 16 c.c. intravenously. There was no apparent effect and the cyanosis continued despite the artificial respiration. The patient died 2 hours later.

CASE 938.—H. K., a boy, aged 12 years. (Patient of Dr. F. E. Rudolf and Dr. L. F. Guldner, Davenport, Iowa.)

Sept. 3.—The child was apparently well until 3 days before, when he felt sick, acted strangely, and was extremely restless. He vomited nearly all of the following night. The next day he had severe pain in the back of the head and neck; the neck was stiff; the temperature was 101. He was tender every- where, did not want to be handled, and was extremely restless. Trouble in swallowing began that night. The temperature was 105. The difficulty in swal- lowing grew steadily worse during the day. He developed great weakness of the muscles of the arms, the neck and the face. 4:30 p. m.—The throat was hyperemic, the tonsils large and infected. One large cervical gland was noted just outside each tonsil and a second gland outside the right. There was com- plete paralysis of the arms, the muscles of the neck, and thorax, and the res- pirations were entirely diaphragmatic. The child choked with every attempt to swallow. There was a mucous rattle in the throat and frothy mucus flowed from the mouth. The extremities were cold; there was clammy sweat on the face; the eyes turned back; there was marked cyanosis; the skin was dusky and mottled; the radial pulse was barely perceptible. The patient continued to be extremely restless and tossed about incessantly in attempting to swallow and to get his breath. A spinal puncture was made, the fluid was under marked pressure and 20 c.c. were obtained. Cell count 320, globulin test + + +. The patient had had diphtheria antitoxin some years previously. Twenty-five hundredths c.c. of serum was given intravenously, followed in 30 minutes by 5 c.c., and in 2 hours by 12 c.c. 7 p. m.—The extremities were warm, the pulse was easily perceptible and regular but rapid. The chest was immobile. 8:30 p. m.—Died.

CASE 942.—C. E., a boy, aged 11 months. (Patient of Dr. R. Dart, Rock Island, Illinois.)

Sept. 6.—The infant was perfectly well until 8 days previously when he had fever which was thought to be due to teething, 3 teeth being in the process of eruption. He had been feverish ever since. The temperature 5 days before was 102.5, 4 days before, 100. During most of this time there was constipa- tion but the last 2 days there was slight diarrhea with green stools. A peculiar staring condition developed the day before. The head was retracted, the eyes were rolled back and he was unable to nurse. Severe convulsions in the morn- ing were followed by difficulty in swallowing. The temperature was 101. 1 p. m.—The left arm and leg were held stiff; the fingers of the right hand were flexed tightly. There was a peculiar staring condition of the eyes. The child was semicomatose; the head retracted and the neck stiff. The tempera- ture was 100, the pulse 110. The tonsils were large; a large amount of pus was expressed from the left. Two enlarged lymph glands were palpable on the left side of the neck just outside the tonsils, but none on the right. The head and neck were held stiff. There was bulging of the fontanelles. He was unable to close the left eye. The left side of the face was partially paralyzed. The knee jerks were exaggerated. He was unable to extend the left index finger and the thumb. The bowels were flushed with a hypertonic solution of sodium chlorid and sodium bicarbonate. A large amount of fecal material

containing a moderate amount of mucus was obtained. A spinal puncture was made, the fluid was under pressure, and 12 c.c. were withdrawn. Cell count 144, globulin test +. The spastic condition of the left arm and leg were not relieved for an hour after the spinal puncture and there was a generalized spasm with retraction of the head 15 minutes afterward. The temperature was 100, the pulse 130. 2 p. m.—Five c.c. of serum were given. Ten minutes after injection of the serum the staring disappeared from the eyes, the left arm and leg relaxed, the baby yawned normally and went to sleep with the eyes completely closed for the first time in 24 hours.

Sept. 7, 9 a. m.—The child rested quietly all the afternoon and evening. The left arm and leg were relaxed. During the night the baby had spells when the arms and legs were set; one severe spasm occurred at midnight and 7 or 8 lighter spasms after that time. The child lay quietly with the eyes open; there was less rigidity of the neck. The fontanelles bulged and pulsated with each pulse beat. A spinal puncture was made, the fluid spurted, and 15 c.c. were withdrawn. Cell count 80, globulin test +. Ten c.c. of serum were given. There was no apparent increase in the paralysis.

Sept. 9, 1 p. m.—The condition was about the same. There was no apparent increase in the paralysis, but there was general spasticity of the legs and arms, and the neck was rigid. The child was semicomatose. Ten c.c. of serum were given. The spastic condition of the arms and legs largely disappeared 30 minutes after the injection of the serum. He moved both legs. 9 p. m.— The condition of the patient was better. The temperature was normal. The spastic condition of the arms and legs had disappeared. He was able to swallow barley water. There were no spasms since the serum was given in the afternoon.

Sept. 10, 2:30 p. m.—The arms and legs were relaxed. There was no retraction of the head. The mouth closed. There was marked exhaustion, the extremities were cold, there was a mottling of the skin and cyanosis; the pulse was 180. 6:30 p. m.—Death occurred, apparently from exhaustion.

Case 943.—E. S., a boy, aged 2 years. (Patient of Dr. E. P. Ficke, Davenport, Iowa.)

Sept. 7.—The child was slightly indisposed incident to the eruption of a tooth 2 weeks previously. He did not appear to have fever and was well until 10 a. m. the day before. Since then he had whined and felt generally miserable. He was restless and hot all night; vomited his supper at 7 in the morning and at 9 a. m. he vomited a large amount of slimy material streaked with blood. He insisted on being held and was very drowsy all the forenoon. 12:15 p. m.— The temperature was 102.3, the pulse 150. The respirations were jerky. The right knee jerk was normal, the left sluggish. He was tremulous and ataxic, scarcely able to walk, and tended to fall to the left. There was marked distention of the abdomen. 5 p. m.—He was very drowsy, could scarcely be aroused; he lay with head retracted, eyes rolled back, mouth open. He was unable to walk, was very ataxic and tremulous, falling to the left, and there was marked weakness of the left leg. The temperature was 103, the pulse 130 and irregular. There was alternate flushing and pallor of the skin. There was paralysis of the left side of the face and he was unable to close the left eye. The arms and legs were tremulous, the knee jerk on the right side was markedly exaggerated, that on the left side was not obtainable. The breathing was jerky. There was no expansion of the upper part of the chest. The respirations were almost wholly diaphragmatic. There was moderate cyanosis. A large amount of mucopurulent material was found in the nasopharynx. The throat was moderately reddened and the tonsils were large. A small amount

of pus was expressed from the pole of the left tonsil. 5:30 p. m.—A spinal puncture was made, the fluid spurted, and 15 c.c. were obtained. Cell count 120, globulin test +. Ten c.c. of serum were given. 8 p. m.—The temperature was 102.2. The bowel movements contained a large amount of mucus. 10:30 p. m.—The temperature was 103. The general condition was about the same. There was marked trembling in the arms.

Sept. 8, 4 a. m.—The parents became greatly alarmed; the child jumped up into sitting position, apparently strangling in an unsuccessful attempt to vomit, and turned white and cold. The bowels moved during this attack and again in 20 minutes, the movements containing a large amount of mucus. The temperature was 103. There was vertical nystagmus. The expansion of the upper chest had returned and the paralysis had apparently not extended. There was less retraction of the head. The child appeared more relaxed and there was less trembling.

Sept. 8, 5:30 a. m.—Six c.c. of serum were given. 11 a. m.—The temperature was 105. There was marked cyanosis and rattling in the throat. 11:30 a. m.—Death occurred from edema of the lungs and respiratory failure.

CASE 945.—V. S., a girl, aged 14 years. (Patient of Dr. L. F. Sullivan, Donahue, Iowa, and Dr. F. Lamback, Davenport, Iowa.)

Sept. 8.—The patient had had an enlarged thyroid for 2 years. About 3 weeks previously an abscessed tooth had caused toothache. A dentist had treated it 3 times and filled it 5 days previously. For a number of days there had been pain in the side of the face and about the ears which was thought to be due to the teeth. Pain in the epigastrium 4 days previously had been relieved with castor oil. She had high fever 4 nights before and was generally ill. The morning before, while drying dishes her legs suddenly became weak and she fell in walking up a few steps, but was able to walk to bed with her mother's help. 2 p. m.—The knee jerks were absent. There was no apparent weakness in the arms. 6:30 p. m.—She appeared quite well while lying in bed, and did not complain of pain. The thyroid was large and firm. There was slight stiffness of the neck, and attempt to flex the head caused pain. There was absence of knee jerks on both sides; marked Kernig and almost flaccid paralysis of legs and arms. When the arms and legs were lifted from the bed, they dropped limply. On examining the tonsils the muscles of the jaw and lips were very tremulous. There was marked photophobia, both vertical and horizontal nystagmus and marked drooping of the eyelids. There was much phlegm in the throat, the tonsils were large, and pus was expressed from the pole of the right. There were 2 small glands outside the right tonsil and one on the left. A spinal puncture was made, the fluid was under moderate pressure and 15 c.c. were obtained. Cell count 130, globulin test +. Five-tenths c.c. of serum was given intravenously, followed in 1 hour by 12 c.c. intravenously. The patient had had diphtheria antitoxin some years previously.

Sept. 9, 5 p. m.—There was no demonstrable increase in the paralysis anywhere and undoubted improvement in the extensors and flexors of the forearms. Twelve c.c. of serum were given.

Sept. 10.—No apparent extension of paralysis.

Sept. 11.—Photophobia was absent. The nystagmus had disappeared. She could rotate the legs and open the eyes normally.

Sept. 15.—There was severe urticaria associated with pain in the extremities and spasms of the muscles of the back.

Sept. 29.—The patient was able to sit up in a wheel-chair. She could bring both hands to her mouth. There was no paralysis of eyes or face. The

grasping power in the arms was quite marked. She had some strength in the lower extremities but was still unable to walk. The knee jerks were absent.

Oct. 16.—There was marked improvement in muscle power, especially of arms, perfect control, except of right deltoid. The power in the lower extremities was gradually returning.

Case 948.—B. W., a girl, aged 11 years. (Patient of Dr. F. Neufeld, Davenport, Iowa.)

Sept. 10, 8 a. m.—The patient was well until the morning before when she awoke with headache and pain in the back of the neck. She did not eat breakfast or dinner. The temperature was 102, the pulse 138. There was tremor of the hands, and the neck was stiff. 2 p. m.—She vomited. The temperature was 103, the pulse 140. 6 p. m.—The temperature was 102, the pulse 145. 9 p. m.—The face was flushed, the head retracted. There was marked photophobia, the eyes were partially closed. The throat was hyperemic, containing a large amount of mucus. The tonsils were enlarged, especially the left, and a large amount of pus was expressed from the pole. There was an enlarged lymph gland outside the left tonsil; none on the right side. While examining the throat the muscles of the face were very tremulous. She was ataxic. The neck was stiff and painful and the right side of the face and the eyelids were partially paralyzed. The knee jerk was active on the right side, but hard to obtain on the left side. Kernig was present on both sides. The temperature was 102, the pulse 145. She was apathetic and drowsy, could be aroused, but immediately went back to sleep. A spinal puncture was made, the fluid was under moderate pressure and distinctly turbid; 20 c.c. were obtained. Cell count 316, globulin test $+\,+$. Six c.c. of serum were given.

Sept. 11, 9 a. m.—She looked brighter. Photophobia was present. The paralysis of the right side of the face was marked. She complained of headache. There was less rigidity of the neck. She was ataxic. Slight weakness was noticeable in the legs when she walked. The temperature was 101 at 2:50 in the morning. White blood count 13,200. 1 p. m.—She had been drowsy most of the forenoon, and still complained of pain in the neck and back. She appeared very sick. There was undoubted extension of the paralysis; photophobia was marked. There was lateral nystagmus of both eyes; she was unable to raise the eyelids completely. The temperature was 101.6, the pulse 124. The right arm was weak, and the grasping power of the hands was diminished on both sides. The expansion of the upper chest was limited. Twenty c.c. of serum were given. Soon after the injection the patient appeared brighter, took interest in her surroundings and said that the headache had disappeared. 6 p. m. —The temperature was 103, the pulse 128. The general condition was about the same. There was no apparent extension of the paralysis.

Sept. 12, 8:45 a. m.—The temperature was 103, the condition worse. There was only slight expansion of the lower portion of the chest, the upper portion was immobile and respirations were almost wholly diaphragmatic. She was cyanotic and seemed anxious and air hungry. Her voice was weak and tremulous. 10 a. m.—The temperature was 102, the pulse 128. The condition was rapidly growing worse. She was comatose and there was marked weakness of both arms. The knee jerks were absent, the plantar reflexes were weak and much delayed. She was unable to lift her legs from the bed in extended position but could flex them at the knees. Extension and flexion of feet were normal. Respirations were diaphragmatic. The abdomen was tympanitic and there was tenderness in the upper half. A spinal puncture was made, the fluid was clear and under slight pressure; 10 c.c. were obtained. Ten c.c. of serum were given.

Sept. 13.—The patient died at 2 : 50 a. m. (Temperature and pulse curves, Chart 3.)

Case 949.—S. C., a girl, aged 17 years. (Patient of Dr. L. F. Newburn, McCausland, Iowa.)

Sept. 11.—The patient was perfectly well until 4 days before, when she had a headache thought to be due to a bilious attack, since she had been subject to such attacks. The following day she vomited repeatedly, she was restless, nervous, and was unable to sleep that night. The next day she felt no better and noticed a peculiar twitching of the muscles. She sat at the table at noon and then went to bed. Several hours later she got out of bed and in attempting to walk fell to the floor and had to be lifted into bed because of weakness of the legs. 6 p. m.—There was almost complete flaccid paralysis of the legs, and marked weakness of the arms. Air hunger and partial paralysis of the muscles of the chest were noted. The temperature was 100.

Sept. 12, 1 : 30 a. m.—The patellar, triceps, and biceps reflexes were absent. The head was retracted, the voice was very weak and tremulous and the respirations labored; there were symptoms of air hunger. The diaphragm was immobile and the excursions of the thorax were limited. She had difficulty in swallowing. There was almost complete flaccid paralysis of the upper and lower extremities; she could just bring the left hand to her mouth. There was marked tremor and twitching of the muscles about the face. The temperature was 101. The throat was hyperemic, the left tonsil was large, the right small. A moderate amount of pus was expressed from the pole of the left tonsil, none from the right. There was a large amount of thick, glairy, mucopurulent material in the nasopharynx. There were 2 enlarged lymph glands on the left side of the neck just outside the tonsil, none on the right side. The thyroid was enlarged. A spinal puncture was made, the fluid was under moderate pressure, was slightly bloody and turbid, and 20 c.c. were obtained. Ten c.c. of unactivated serum were injected intraspinally and 25 c.c. intravenously. 6 : 30 p. m.—She was comatose, the head was retracted, and she was markedly cyanotic. There was a mucous rattle in the throat. The excursion of the thorax was slight, the diaphragm immobile. There was a relaxation of the abdominal walls. 7 p. m.—The patient died.

Case 955.—W. N., a boy, aged 13 months. (Patient of Dr. H. U. Brannlich, Davenport, Iowa.)

Sept. 13.—The child had been perfectly well until 2 a. m. 3 days before, when he vomited, had high fever and was cross and fretful. The fever continued for 3 days and the condition was thought to be la grippe, since other members of the family had had similar attacks. The afternoon before he had been unable to sit up and constantly fell forward. There was marked weakness of the muscles of the neck and the legs. 9 p. m.—The temperature was 99.8. There was flaccid paralysis of the lower extremities and marked weakness of the muscles of the back, the neck and the left arm. He was fretful and irritable and when handled appeared to be in pain. The tonsils were rather large. There was a large lymph gland on the left side of the neck outside the tonsil, none on the right side. A spinal puncture was made, the fluid was under pressure, and 20 c.c. were obtained. Cell count 133, globulin test + +. Six c.c. of serum were given.

Sept. 14.—The temperature was normal and there was less restlessness and no extension of paralysis, otherwise but little change.

Oct. 15.—Marked improvement. The child was able to stand erect.

Case 957.—L. S., a girl, aged 9 months. (Patient of Dr. S. G. Hands, Davenport, Iowa.)

Sept. 14.—The child became ill 5 days before with fever and vomiting. The temperature the second day was 99.2. She appeared drowsy and slept most of the time with head retracted and eyes partly open. The left leg was weak for two days. 10 : 30 a. m.—The left leg was almost completely paralyzed. The right eye was turned in and there was marked weakness of the muscles of the neck and back and she was unable to hold up her head or sit up. The tonsils were large. The throat was diffusely hyperemic with profuse secretion in the pharynx. There was 1 lymph gland on either side just outside the tonsil. A spinal puncture was made, the fluid was under pressure and 10 c.c. were obtained. Cell count 30, globulin test + +. Six c.c. of serum were given.

Sept. 15.—There was no extension of paralysis.

Oct. 15.—Marked improvement was noted soon after administration of the serum.

Case 959.—D. C., a girl, aged 12 months. (Patient of Dr. C. C. Sloan, Moline, Illinois.)

Sept. 14.—The child was taken sick 4 days previously with high fever, vomiting and diarrhea. The abdomen was distended, the stools green and of foul odor. There was marked depression and a tendency to sleep constantly with the head retracted. Paralysis of the lower extremities appeared the day before, and difficulty in swallowing began in the morning. 4 p. m.—There was complete flaccid paralysis of the lower extremities, the knee jerks were absent. She was comatose and cyanotic, there was a mucous rattle in the throat, and she was unable to swallow. The upper portion of the chest was paralyzed. She was restless and the eyeballs and muscles of the hands twitched. The extremities were cold. There was retraction and rigidity of the neck. The temperature was 104, the pulse was not obtainable. A spinal puncture was made, the fluid was under pressure and 10 c.c. were withdrawn. Cell count 58, globulin test + +. Six c.c. of serum were given. 5 p. m.—The restlessness had disappeared; she had fallen asleep with eyelids closed. The twitching of the hands had disappeared. 10 p. m.—The patient died of respiratory failure.

Case 960.—E. S., a girl, aged 16 years. (Patient of Dr. C. F. Cron, Long Grove, Iowa.)

Sept. 15.—The patient had had severe headache, fever, and dizziness with repeated vomiting the afternoon before, in the night and almost constantly during the next forenoon. There was marked weakness of the legs; paralysis of the left side of the face, and external strabismus of the left eye. Inability to swallow began in the night. 9 a. m.—The voice was husky and tremulous. The patient tossed about in attempts to swallow. She vomited a large amount (fully 2 quarts) of mucus containing discolored blood, at frequent intervals. There was cyanosis, intense thirst, air hunger, and she was entirely unable to swallow. The eyes turned sharply inward, the head was retracted, and the neck stiff. Kernig +. The knee jerk on the left side was normal, but absent on the right side. There was a large amount of secretion in the pharynx. The tonsils were of moderate size; a palpable lymph gland was noted on either side. Marked Babinski. There was complete paralysis of the left side of the face. She was unable to open her mouth more than 1.5 cm. There were marked weakness of the extensors of the left arm. Expansion of the chest was limited. The temperature was 103. A spinal puncture was made, the fluid was under increased pressure and distinctly turbid, and 15 c.c. were obtained. Cell count 344, globulin test + + +. Twelve and one-half c.c. of serum were given. The

patient was quieter for several hours after the injection then became restless again and died 6 hours later of respiratory failure.

CASE 963.—M. S., a boy, aged 3 years. (Patient of Dr. D. G. Kreul, Davenport, Iowa.)

Sept. 16.—The child was restless and irritable and unable to sleep 4 nights before. The next day he complained of pain in the throat, he was irritable and feverish in the morning, with high fever at night. The following night he vomited 3 times, and was restless and unusually drowsy. The next day there was marked jerking of the muscles and weakness of the muscles of the neck and arms developed during the night. 11:50 a. m.—The child was extremely ill. His face was alternately flushed and pale, and he lay with head retracted and eyes turned in sharply. There were marked twitchings of the eyeballs. He cried out at frequent intervals and threw himself from side to side. Attempts to flex the head caused pain. Kernig marked. He was tremulous and unable to stand because of weakness of the legs. The right knee jerk was obtainable, the left absent. He could not hold up his head. He had great difficulty in swallowing and there was a mucous rattle in the throat. The muscles of the chest apparently were not affected. Both arms were very weak and he was unable to bring his hands to his mouth. There were coarse râles over the chest. He was semicomatose. The temperature was 103.5. A spinal puncture was made, the fluid was under increased pressure, clear, and 3 c.c. were withdrawn. Cell count 277, globulin test + + +. Ten c.c. of serum were given. 12:22 p. m.—The child was quieter. Nystagmus and strabismus were less marked. 8:30 p. m.—The child appeared brighter and was undoubtedly better. The twitching of eyeballs and the strabismus were gone. The color was good. He could swallow without difficulty. Respirations were normal, the head less retracted, the face symmetrical. The muscles of the neck were undoubtedly stronger but there was no change in the weakness of the arm. Six c.c. of serum were given.

Sept. 17, 12 m.—He slept quietly. The retraction of the head was less marked and the alternate flushings and pallor had disappeared. The pupils were equal. There was no strabismus. Excursions of the chest were normal. The pupils were equal. He was interested in the surroundings. The left knee jerk was barely obtainable, the right slightly plus. Kernig less marked. The muscles of the neck were stronger, the arms were weak. There was no extension of the paralysis. Six c.c. of the serum were given.

Sept. 18, 12 m.—The child slept well the night before. His color was good. He was able to hold his head erect when in a sitting position. He had no difficulty in swallowing and could bring his left hand to his mouth. The knee jerk on the right side was normal, on the left side diminished but obtainable. Five c.c. of serum were given.

Sept. 20.—The child was much brighter. The right eye tended to turn inward at intervals, the face was symmetrical, there was no difficulty in swallowing, the head was held erect when in sitting position. He could move his arms in every direction but there was slight weakness. The knee jerks were normal.

Oct. 15.—There was marked improvement in muscle function.

Nov. 8.—Complete recovery assured.

(Temperature and pulse curves, Chart 3.)

CASE 967.—L. B., a girl, aged 7 years. (Patient of Dr. M. S. Jordan, Clinton, Iowa.)

Sept. 17.—Two weeks before the child became ill with vomiting, projectile in character, high fever, and severe headache. She was extremely restless, and

tremulous. She was mentally apathetic, drowsy, and then semicomatose with retraction of the head. The extremities were spastic. 11:45 p. m.—The temperature ranged from 99 to 103. She lay in bed, comatose, with the head retracted, and could not be aroused. Tache cérébrale was marked. Knee jerks were absent. Marked Kernig and Babinski signs. The muscle tonus of the arms was increased. There was almost complete flaccid paralysis of the right leg, and combined flaccid and spastic paralysis of the left. The tonsils were normal in size, and there were enlarged cervical glands outside of both tonsils. There was much mucus in the pharynx. A spinal puncture was made, the pressure was not increased, and 10 c.c. of clear fluid was obtained. Cell count 50, globulin test + +.

Sept. 18, p. m.—The patient was comatose and weaker. Ten c.c. of unactivated serum were given.

Sept. 19.—The patient died.

CASE 978.—F. E., a girl, aged 3½ years. (Patient of Dr. P. H. Wessel, Moline, Illinois..

Sept. 24.—Four days previously the child had had headache, pain in the back of the neck, twitchings of the muscles and high fever. The temperature was 103.5, the pulse rapid. The fever continued high for 3 days. Weakness of left leg was noted 2 days before, more marked the following day. 4:30 p. m.—There was almost complete flaccid paralysis of the right arm and left leg and the muscles of the neck were weak. The tonsils were diffusely red, and pus was expressed from the pole of the left. There were 2 cervical glands outside the left tonsil, none on the right side. The temperature was 99, the pulse 120. A spinal puncture was made, the fluid was under pressure and 10 c.c. were obtained. Cell count 83, globulin test + +. Ten c.c. of serum were given.

Sept. 25.—There was no extension of paralysis. The temperature and pulse were normal.

Oct. 15.—The right arm and left leg were still weak.

CASE 980.—W. K., a boy, aged 15 months. (Patient of Dr. L. J. Porstman, Davenport, Iowa.)

Sept. 24.—The child had recently recovered from whooping-cough. He had been generally ill, with fever, vomiting, and fretfulness for the past 3 days. Tremors and jerky movements of various muscles during sleep had been noted from the beginning of the illness. There was marked weakness of the muscles of the neck, and difficulty in swallowing was noted the day before. 4 p. m.— The muscles of the neck were completely paralyzed. The voice was extremely weak. The thorax was immobile, and the respirations were wholly diaphragmatic. Expansion of the diaphragm on the left side was limited; there was marked pallor and he was unable to swallow. A marked weakness was present in the arms and in the left leg. The temperature was 100.6. He was stuporous. A spinal puncture was made, the fluid was slightly turbid, and 10 c.c. were obtained. Cell count 347, globulin test + + +. Twelve c.c. of serum were given.

Sept. 25, 9 a. m.—The child was undoubtedly better, brighter mentally, the voice was stronger, and he could move his head from side to side. There was definite expansion of the chest. The temperature was 99.6, the pulse 105. 1:30 p. m.—The condition was much changed. There was cyanosis, mucous rattle in the throat and coarse mucous râles over the right lung posteriorly. The respirations were 60 per minute. The temperature was 103, the pulse was extremely rapid.

Sept. 26, 9 a. m.—The patient died.

CASE 983.—G. F., a girl, aged 9 months. (Patient of Dr. F. O. Ringnell, Rock Island, Illinois.)

Sept. 25.—The child was cross, restless, irritable and feverish 2 days before but felt quite well the day before. Between 9 and 10 p. m. the mother found that she could not hold up her head. There was marked jerking and tremor of the muscles of the left side of the body. Similar attacks occurred during the night. In the morning the left leg was weak and she was unable to move the right arm and the right leg. The temperature was 102.8. 4 p. m.—The head was retracted. She did not move the right arm and the right leg and they fell limply. The knee jerk on the left side was exaggerated, and absent on the right side. The eyes turned to the left. A spinal puncture was made and 12 c.c. of fluid were obtained; the fluid was bloody but this was traumatic, due to difficulty in getting into the spinal canal. Six and one-half c.c. of serum were given.

Sept. 26, 3 p. m.—The mother said the baby rested quietly all the afternoon and night. In the morning the temperature was 102.5. She moved the right hand and leg, the latter with considerable strength. There was less rigidity of the neck. Attempts at flexion still caused pain. She looked brighter, the temperature was normal; 5 c.c. of serum were given. There was no extension of paralysis.

Oct. 15.—Marked improvement.

CASE 985.—J. B., a boy, aged 3¾ years. (Patient of Dr. P. H. Wessel, Moline, Illinois.)

Sept. 26.—Fever had developed during the night 4 days before. The child vomited the following evening, was constipated and had headache. There was jerking of various muscles of the body, pain in the right leg, and fever. He was very drowsy. Three days later (Sept. 26) there was marked weakness of the right leg. 2 p. m.—He complained of pain in the right leg, and was unable to walk. The temperature was 100, the pulse rapid. A spinal puncture was made and 10 c.c. of fluid were obtained. Cell count 222, globulin test + + +. Ten c.c. of serum were given.

Sept. 27.—The temperature went to 102 during the night. In the morning the temperature was normal. The paralysis was not extended. Definite improvement had occurred in the power of the right leg.

Oct. 15.—Some weakness of the right leg was still present.

CASE 995.—I. R., a girl, aged 11 years. (Patient of Dr. L. F. Sullivan, Donahue, Iowa.)

Sept. 27.—The girl broke her right arm in a fall 5 days before. She felt ill, had a headache and some fever. There had been pain in the left leg for 2 days, thought to be due to the fall. 7 p. m.—The weakness of the left leg was marked and the neck was slightly rigid. The knee jerks were absent. The temperature was 100.

Sept. 28, 2:30 a. m.—There was marked flaccid paralysis of legs and arms, she could barely rotate the left leg and was unable to lift it in extended position. She was just able to lift the right foot from the bed with the knee partly flexed. She could not flex the left leg at the knee. There was marked weakness of the extensors and flexors of the left forearm and undoubted weakness of the right arm, but accurate tests could not be made on account of the fracture of the forearm. She could move the muscles of the arm but with very little strength. The knee jerk and plantar reflex were absent on the left side; on the right side they were delayed but obtainable. A spinal puncture was made, the fluid was under moderate pressure and 15 c.c. were obtained. Cell count 97, globulin test + +. Twenty c.c. of serum were given. 5:30 p. m.—No

extension of paralysis had occurred. The right leg was possibly a little weaker. The grasping power of the left hand was undoubtedly greater than the night before. She could move her arms in all directions. Fifteen c.c. of serum were given.

Sept. 29.—Grasping power of the left hand was increased. The power of extension at left wrist was increased. The power of biceps, triceps, and deltoid muscles was also increased. The knee jerk on the right side was obtainable, on the left absent. Plantar reflex on left side was obtainable. There was undoubted improvement in the strength of the right leg, but no change in the left. Ten c.c. of serum were given.

Oct. 16.—There was marked improvement, complete restoration of muscle power except in left leg, which also showed improvement.

Case 1008.—C. G., a girl, aged 11 months. (Patient of Dr. G. T. Joyce, Rochester, Minnesota.)

Oct. 12.—Three days previously the child vomited and had a severe convulsion which lasted 4 hours. This was at first attributed to teething. She was extremely nervous and restless, and had jerky spells and twitchings of the muscles during the night and forenoon following the convulsion. She then became listless and drowsy, took no notice of things and slept most of the time. A doubtful weakness of the right arm first noticed 24 hours previously. 12 m.—There was undoubted weakness of the right arm and right leg and the muscles of the right side of the face. She could move both the right arm and the right leg but her strength was diminished. The knee jerk on the right side was diminished, on the left side normal. There was a tendency to fall to the right when she sat up. The neck was rigid and attempts to flex the head caused pain. 2 p. m.—There was undoubted extension of the paralysis. The right side of the face was more drawn, and there was no power whatever in the right arm and right leg. She was not able to pull the arm or leg away when the skin was pricked in giving the serum. She continued listless and took little notice of things. The tendency to fall to the right when sitting was more marked. The spinal fluid was clear and 2 c.c. were obtained. Cell count 8, globulin test + +. Ten c.c. of activated serum were injected intravenously. 7 : 30 p. m.—The right side of the face was less drawn. She appeared brighter, sat erect without falling to the right, and moved the right arm and leg.

9 : 30 p. m.—Improved. Ten c.c. of serum were given. The child had considerable power in the right forearm and succeeded in dislodging the needle from the small vein at the wrist in spite of the fact that the arm was held by a trained assistant. There was marked power in the right leg, making intravenous injection difficult.

The improvement after the injection of the serum was gradual. One month afterward the restoration of function of the muscles of the right leg, back, and the right side of the face was complete. Slight weakness of right hand was still present but has since disappeared.

Case 1024.—N. F., a boy, aged 2 years. (Patient of Dr. M. Bachman, Lake Park, Iowa.)

Nov. 3, 1917.—The illness began 2 weeks before with high fever, coryza and a moderately sore throat. He had been constipated but did not vomit. He was hoarse and had fever for 4 days. The temperature was then normal. Marked weakness of the legs, especially the right, and the back began on the 4th day. Weakness of the right arm was first noted on the seventh day. There was little or no improvement, he could just stand but cried from pain when made

to take a step with support. He was pale, still very restless, cross and irritable, and complained of pain in the legs. He cried out while asleep and was unable to roll over in bed. Pus was expressed from the left tonsil which was larger than the right. There were enlarged paratonsillar glands on the left side, none on the right. There was marked weakness of the right leg, the right knee jerk was barely obtainable, the left normal. He could move the right arm in all directions but with impaired power. 4 p. m.—The spinal fluid was under pressure, clear, and 8 c.c. were obtained. Cell count 16, globulin test $+ +$. Twelve c.c. of unactivated serum were injected intravenously.

Nov. 5.—The parents stated that the child slept soundly without waking for 5 hours following the injection of the serum. The pain had disappeared the day before. While lying down he kicked a hand with the leg in the extended position. He could walk and roll over in bed. He looked brighter and was more contented. The right knee jerk was more active.

The improvement has continued, and complete recovery will probably take place.

<div align="center">RESULTS</div>

Group 3.—*Patients showing advanced paralysis at the time of serum treatment.* In 25 patients the serum treatment was begun after advanced paralysis had already occurred. Of these 10 died, a mortality of 17%. However, in 7 of the fatal cases (Cases 937, 938, 949, 959, 960, 967 and 980) the patients were moribund or markedly cyanotic from respiratory paralysis when the treatment was begun. Four of these (Cases 938, 959, 960 and 980) showed temporary improvement following the serum injection. In the other 3 fatal cases (Cases 942, 943, and 948) there was sufficient time for the serum to act. In all three temporary improvement followed the injection of one or more doses of serum. One of these (Case 942) was a baby 11 months old, semi-comatose with spasms 8 days after onset of the illness when the serum was first given. One (Case 943) was a boy, 2 years of age, with cyanosis, marked tremors, high fever, severe gastro-enteritis and beginning respiratory paralysis on the second day, at the time of the first injection. The other (Case 948) a girl, 11 years of age, had a fever, paralysis of the face, marked photophobia, ataxia, tremulousness, increased respiration and diminished knee jerk on the left side, on the third day, when the first dose of serum was given. The last 2 showed numerous characteristic abscesses in the tonsils containing the pleomorphic streptococcus in enormous numbers.

In the 15 patients who recovered, the paralysis did not extend perceptibly and in 8 (Cases 931, 932, 933, 934, 945, 963, 998, and 1008) a progressing paralysis appeared to be arrested. Marked early improvement as manifested by the drop in temperature and pulse rate and by restoration of muscle power or disappearance of other symptoms was

noted in 10. In 1 patient (Case 933) there was already moderate cyanosis from beginning paralysis of the muscles of the thorax when the serum was first given, followed by prompt early improvement and almost complete recovery.

The ages of the patients in which there was advanced paralysis ranged from 9 months to 17 years, the average being 6 years. The average age of the 15 patients who recovered was 4 years; that of the 10 patients who died was 7 years. The spinal fluid was under pressure in all but 2 patients (Cases 935 and 1008), it was clear in the 15 patients who recovered and turbid in 4 of the 10 who died. The amount withdrawn ranged from 3-30 c.c., the average being 13 c.c. The cell count ranged from 0-347. The average in the nonfatal cases was 104, in the fatal cases 209. The average for both was 156. The globulin content was increased in all but 1 patient in whom the puncture was made 2 weeks after paralysis had occurred. In one instance no fluid was obtained. The temperature of 4 patients was not taken at the time of the injection, in two it was normal, in the others it ranged from 99-104. The pulse was relatively rapid in most instances. The serum treatment was begun on the first day in 2 cases, on the second day in 4, on the third day in 6, on the fourth day in 8, on the fifth day in 1, on the eighth day in 1, and on the fourteenth day in 3. Only 1 dose was given in 14 cases, 2 doses in 4, and 3 doses in 7. The amounts given in each case ranged from 6-36 c.c., the average being 17 c.c.

Complete restoration of function has occurred or is already assured in all but 5 patients in this group. In these patients (Cases 935, 945, 978, 995, and 1008) paralysis was extensive when the serum was first given and had existed 14, 7, 3 days, 1 day, and 1 day respectively. Improvement in these patients occurred in proportion to the promptness of the serum treatment. Thus in the patients (Cases 935 and 945) to whom the serum was given on the 14th and 7th days, respectively, improvement was slow, while in the others it was very marked, especially in the patient (Case 995) in whom the serum was given within 24 hours after the onset of rapidly progressing ascending paralysis, as was also true in the patient (Case 1008) with unilateral paralysis.

Cases in which Serum Treatment Was Not Given

The following protocols are illustrative of this group: *

CASE 1.—A. G., a young man, aged 26 years. For six days there had been vomiting and diarrhea with a temperature of 101-102. Four days previously

* These data were obtained through the courtesy of Dr. Rendleman.

there was weakness of the left leg and arm, soon followed by flaccid paralysis of the left arm, leg and face.

Aug. 13.—The patient was unconscious, cyanotic and the breathing was stertorous. The temperature was 101. There was no rigidity of the neck, and no Kernig. A spinal puncture made August 10 showed some increased pressure, cell count of 180 and globulin test +.

Death from respiratory failure occurred August 14, 7 days after the onset of ascending paralysis.

CASE 2.—J. A., a boy, aged 8 years. The illness began during the day August 18 with fever and vomiting. 7 p. m.—He vomited every few minutes. The temperature was 104.2 by axilla. He was constipated and the bowel movement after an enema was very foul. The vomiting continued through the night.

Aug. 19, 5 a. m.—Axillary temperature 105, pulse 160. He was cyanotic, the respiration was short and rapid and he was unable to swallow. Attempts to swallow water caused strangulation and convulsion. There was marked opisthotonos during the convulsion. He resisted having the head lifted forward, but the rigidity was not constant, the muscles relaxed at times and the head came forward easily. He was semicomatose. The temperature reached 106 and the pulse was above 160. He vomited a large amount of coffee-ground material.

Death occurred from respiratory paralysis August 19, 24 hours after the onset.

CASE 3.—G. R., a boy, aged 2 years.

Aug. 21.—The child awoke with some fever. He was constipated and vomited once at noon. The temperature was 102, the pulse 140. He lay on his back and objected to being disturbed. There was tremor of the extremities, and he walked with a limp in the left leg. He complained of pain when the left leg and the head were moved. The left patellar reflex was absent, the right present; both plantar reflexes were present.

Aug. 22.—The temperature was 101.5, the pulse 140; breathing rapid. Paralysis of the left lower extremity was almost complete.

Aug. 23.—The temperature was 101-102. He was very restless, and cried out when moved. The pulse was rapid, the breathing rapid and short. The left leg was completely paralyzed, patellar and plantar reflexes of the right leg gone, but motion was still present.

Aug. 24.—Both lower extremities were completely paralyzed. There was very slight movement of the toes of the right foot when the sole of the foot was stroked. Urination was frequent. He was unable to hold his head up when it was raised.

Later history.—The fever continued 100-101 for 6 days after the paralysis was complete. The pulse was above 120 for 4 weeks, then gradually returned to normal. For 3 weeks he was restless and cried when moved. He slept very little at night.

Oct. 17.—To date there had been no improvement of either lower extremity. There was absolutely no motion in the left and only the minutest movement of the toes of the right foot with stroking of the plantar surface. He could with difficulty hold the head erect when the body was supported.

Nov. 7.—Death occurred from pneumonia.

CASE 4.—J. G., a boy, aged 5 years.

Oct. 8.—The temperature the evening before was 102. The child vomited and was constipated. He was not seen by a physician until 3 p. m., when he was semiconscious and cyanotic. The respiration was 60 and stertorous. He was restless and resisted having his head lifted forward. The patellar reflexes

were gone, the plantar and triceps reflexes were present. There was no paralysis but a spastic condition of the upper extremities. The temperature was 103, the pulse 160. During the examination the patient became distinctly worse. No sign of cranial nerve involvement could be detected but because of the mental condition it was impossible to tell whether or not he could swallow or talk. A spinal puncture showed the fluid under slightly increased tension. Cell count 150, globulin test negative. A general examination failed to disclose any trouble in the chest or abdomen. The tonsils had been removed 2 weeks before.

Death occurred at 6 p. m., 3 hours after the diagnosis was made and 24 hours from onset of symptoms.

About 1 month previously I saw this patient as a suspected case with Dr. Weber. The symptoms at that time were due to tonsillitis, the attack resembling previous similar attacks.

RESULTS

GROUP 4.—*Cases which occurred during the same epidemic but in which the serum treatment was not given.* Altogether there were 23 cases classified in this group. Nine of these patients died, a mortality of 35%. If the 7 fatal cases were included in which the patients were moribund at the time of the serum treatment, there would be 16 deaths in the 30 cases, a mortality of 53%. Complete data were not obtained in all of these cases, but in that available it was shown that the average age of the patients was 5 years; the onset was practically the same as in those who were treated; it was often acute, with high fever, rapid pulse, and severe gastro-intestinal symptoms. The incidence of paralysis in this group of patients was 100%. Improvement in paralysis in those who lived was slow and slight as compared with those who received the serum treatment.

DISCUSSION AND SUMMARY

Intravenous injections were made to the exclusion of intraspinal injections for the following reasons:

1. The best results were obtained by this method in the protection of monkeys against virus. The serum was activated for the same reason.

2. Invasion of the nervous system in poliomyelitis is only a part of a more or less generalized systemic infection (Flexner and his co-workers). This was particularly true in this epidemic. Infection of the tonsils, the cervical and mesenteric lymph glands and the gastro-intestinal tract was often marked.

3. The spinal fluid in poliomyelitis is known not to contain the virus. The disease process is situated chiefly in the depths of the cord which can best be reached through the circulation, particularly if lymph

drainage towards the spinal canal is promoted by the withdrawal of spinal fluid.

4. Intraspinal injections of immune serum, human and horse, are known to be irritating and at times dangerous. They may produce, to quote Draper, "severe pictures of meningeal irritation, with vomiting, opisthotonos and sometimes convulsions." Peabody warns against intraspinal injections in patients who have already developed paralysis. Moreover, intraspinal injections of serum increase the susceptibility of monkeys to intravenous inoculation of virus (Flexner and Amoss). Hence, any good which follows intraspinal injections of immune serums occurs in spite of these primarily undesirable and at times probably harmful effects.

Altogether, 94 intravenous injections were made. In no instance was there a primary toxic action noticeable, and in only 6 (10%) was there later evidence of serum disease. If the temperature was normal no rise occurred, if above normal, an immediate drop without an initial rise was the rule, especially, early in the disease. In this respect the action of the serum differed from that following intraspinal injection of immune human (Zingher, Amoss and Chesney, Draper, and Peabody) or immune horse serum (Nuzum and Willy) when, owing probably to the toxic action of the serum on the meninges there is often first an initial rise in temperature and then a drop.

The low incidence of serum disease in my series, 10%, as compared with the incidence of 33% in Nuzum and Willy's series, and apparently a more immediate beneficial effect, may be due (aside from an apparently more powerful serum, the agglutinating power being much higher, smaller doses being necessary to be effective) to the fact that intravenous injections only were given.

The exact mode of action of the serum is not definitely known. However, it is probably specific in nature and not due to nonspecific effects, because normal horse serum and the serum of Horse 3 (with and without cresol) injected with strains which had lost their specific antigenic properties had little or no protecting power against virus in monkeys.

Altogether 58 patients with poliomyelitis, irrespective of the severity or type of the disease, were treated. Of these 10 died, a total mortality rate of 17%. Excluding 7 of the fatal cases in which the patients were practically moribund at the time of the serum treatment, there were 3 deaths, a mortality of 6%, in 51 cases in which the serum had a fair

chance. This is in marked contrast to the 23 untreated cases, in which
9 patients died, a mortality of 35%. Including the moribund patients
as untreated, there were 16 deaths in 30, or a mortality of 53%. That
the patients treated in the early stages were undoubted cases of polio-
myelitis is indicated by the symptoms, the increased pressure of spinal
fluid, the cell count, the positive globulin test, and by the fact that in 2
cases not poliomyelitis in which spinal puncture was done no cells were
found in the spinal fluid, and the globulin test was negative. One of
these was an emaciated baby 9 months old with fever, vomiting, per-
sistent diarrhea, green stools, and repeated convulsions. The other
was a girl 5 years of age with severe headache, high fever, vomiting,
full, rapid and bounding pulse, and enlarged, acutely infected tonsils
from which cheesy plugs were expressed. Moreover, the findings in
these 2 cases are in accord with those of Wells, who has found few
or no cells and no increase in globulin in the spinal fluid of patients
presenting symptoms in common with acute poliomyelitis.

Paralysis did not develop in a single instance when treatment was
begun before its onset, and all recovered. According to Draper (cited
by Peabody) about 50% of proved cases develop paralysis if untreated.
A comparison of the results of the treatment of preparalytic cases by
immune human and immune horse serum is of interest in this connec-
tion. Eighteen per cent of 54 patients treated by Zingher with immune
human serum developed paralysis with no deaths. Twenty-nine per
cent of the 14 patients treated by Amoss and Chesney developed paraly-
sis and 14% died. Thirty-one per cent of 51 patients treated in
Peabody's series developed paralysis and 10% died. None of 14 cases
treated by Nuzum and Willy developed paralysis but 1, or 7%, died.
In my series of 16 preparalytic cases none developed paralysis and
none died.

No extension occurred following the giving of serum in the patients
who recovered and in whom paralysis was marked at the time of the
serum treatment. In only 3 or possibly 4 patients, or 8%, receiving
the serum will there be permanent impairment of function, and in all
but one this will be slight. Wickman's 530 patients showed residual
paralysis in 56% one and one-half years later. Massachusetts records
cited by Draper show a permanent paralysis of 83%. The good effect
was not due wholly to withdrawal of spinal fluid because the amount
removed was relatively small in all cases and too small to have any
possible effect in a number in each group which showed striking
improvement.

The drawing of conclusions as to the exact value in this disease of any treatment is most difficult. Considering all the facts, however, the serum used appeared to have a prompt and powerful beneficial effect in a very large percentage of the patients treated. Its harmlessness, at least, is demonstrated and its use on a large scale indicated. The treatment should be given before paralysis has developed, hence early diagnosis by spinal puncture should be made. The course of this disease should be considered in terms of hours, not days, particularly now that there is available what appears to be a curative serum. The serum is of distinct benefit at least as long as postparalytic pains are present or the spinal fluid is positive. The strikingly favorable result Case 1008 indicates that the serum will be of value in the treatment of sporadic poliomyelitis.

The fact that so many patients recovered completely in such a remarkably short time following injection of this serum indicates that the pleomorphic streptococcus is not merely a secondary invader but is in some way, as yet partially obscure, the cause of epidemic poliomyelitis.

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IMMUNITÉ ET ANAPHYLAXIE

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En analysant l'ensemble des travaux accumulés jusqu'à présent sur l'immunité et l'anaphylaxie, on peut en dégager un certain nombre de faits que nous allons résumer, aussi brièvement que possible, tout d'abord avec les interprétations qui en ont été données.

Ensuite nous verrons s'il est possible de dégager, dès à présent, de cet ensemble complété par quelques expériences nouvelles, une idée générale plus complète et plus claire que celles actuellement en cours, pour expliquer la nature et le mécanisme des réactions qui déterminent les différentes phases de l'évolution des maladies infectieuses, des états pathologiques causés : par les albumines animales ou végétales considérées comme normalement toxiques, telles que les venins, les sérums d'anguille et de muréne, la ricine, l'abrine, etc ; par les colloïdes synthétiques, tels que les arsenobenzènes et enfin, par les albumines considérées comme exclusivement nutritives, quand elles pénètrent dans le milieu intérieur de l'organisme par l'intestin après avoir subi une digestion gastro-intestinale complète, mais qui provoquent l'état anaphylactique, quand on les fait pénétrer dans ce milieu par des injections hypodermiques, intraveineuses ou même intrarectales.

On n'a pas manqué de remarquer déjà combien il est difficile de bien démarquer les limites entre les différents groupes de substances que nous venons d'énumérer. Ainsi, un certain nombre d'albumines toxiques et même de microbes pathogènes peuvent être absorbés par la bouche sans provoquer aucun trouble, d'autres restent pathogènes après avoir subi la digestion gastrique et intestinale. Toutes possèdent un certain nombre de caractères communs, et la valeur de ces caractères pour une classification quelconque fournira toujours une matière commode à des discussions interminables.

Il faut donc avoir constamment présent à l'esprit que les distinctions que nous avons indiquées plus haut, et que nous ferons encore, n'ont pas de valeurs absolue, qu'elles ne sont que provisoirement indispensables pour la clarté de l'exposé que va suivre.

On a donc constaté les faits suivants :

1° *Les toxines tétanique et diphthérique* peuvent être injectées à des animaux sensibles à des doses non toxiques ou toxiques, et alors :

(a) Chaque injection d'une dose non toxique ou peu pathogène protège l'animal injecté, après un certain temps d'incubation, contre une dose plus pathogène pour un animal neuf : *immunité active*.

(b) Le sérum de l'animal ainsi *activement immunisé* neutralise l'action pathogène de la toxine *in vitro* et injecté préventivement à un animal neuf protège cet animal contre une dose pathogène : *immunité passive*.

(c) Les injections des toxines, des mélanges des toxines avec leurs antitoxines, ainsi que les injections préventives des sérums "anti" à des animaux neufs, ne provoquent jamais de troubles anaphylactiques.

Donc pour les toxines tétanique et diphthérique, il y a immunité active et passive, et il n'y a jamais d'anaphylaxie active ni passive.

2° *Pour les microbes pathogènes vivants,* il y a très probablement aussi des doses non pathogènes ; mais dans la pratique des vaccinations, il est plus commode d'employer des cultures vivantes d'une virulence atténuée, des corps microbiens tués par la chaleur ou par d'autres procédés, ou encore des bouillons de culture filtrés, dépourvus de microbes.

L'injection de tous ces produits en doses non pathogènes provoque, d'une part, des réactions de la même nature que dans le cas précédent, c'est à dire :

(a) *Une immunité antiinfectieuse active* à l'animal traité.

(b) Le sérum de l'animal traité confère *une immunité passive* aux animaux neufs :

mais en même temps, on constate que le même traitement a provoqué ;

(c) Un *état d'anaphylaxie active* à l'animal traité, et

(d) que le sérum de cet animal est devenu *passivement anaphylactisant* pour un animal neuf.

Donc, pour tous les microbes pathogènes vivants ou morts, ainsi que pour leurs bouillons de culture filtrés, il y a, à la fois et en même temps, immunité antiinfectieuse active et passive et anaphylaxie active et passive.

3° Les albumines pathogènes se comportent au point de vue de leurs réactions immunisantes et anaphylactisantes exactement de la

même façon que les microbes pathogènes, et il est naturel que cela doit être ainsi, parce que les microbes ne peuvent agir que par leurs produits de sécrétions ou de bactériolyse solubles.

Donc, pour les albumines pathogènes, il y a à la fois immunité antitoxique sensibilisation anaphylactique active et passive.

4° *Pour les albumines hétérogènes exclusivement nutritives, il ne peut évidemment être question d'immunité active ou passive, mais il y a anaphylaxie active et passive.*

Toutes ces substances ont donc une propriété commune : leurs injections à un animal neuf en doses non pathogènes ou pathogènes provoquent toujours une réaction de la même nature : la formation d'un anticorps qui est toujours spécifique, sans être toujours rigoureusement et exclusivement spécifique.

On a donc groupé toutes ces substances sous le nom *d'antigènes,* et on a donné le nom *d'anticorps* aux substances qui se forment dans l'organisme sous l'action de ces antigènes et possèdent une affinité spéciale, sinon toujours exclusive pour leurs antigènes respectifs.

Quand on a cherché à définir les propriétés physicochimiques des antigènes, on a reconnu que ce sont toujours des albumines ou des colloïdes ; quant aux anticorps, on n'a jamais pu les isoler des albumines des sérums dans lesquels on les trouve ; on doit les considérer aussi comme des colloïdes, ou tout au moins considérer leur action comme une fonction des colloïdes.

Mais alors, on a reconnu aussi que toutes les albumines ne sont pas "antigènes", que cette propriété n'appartient qu'aux albumines dites "hétérogènes" et que les albumines "homogènes", c'est à dire provenant d'individus de la même espèce, ne provoquent pas de formation d'anticorps et ne sont pas anaphylactisantes. Et on doit en conclure que la réaction qui provoque la formation des anticorps et qui confère l'immunité seule ou, à la fois l'immunité et l'anaphylaxie, ou enfin, l'anaphylaxie seule, n'est pas déterminée uniquement par la nature physico-chimique des antigènes, puisque les albumines homogènes sont à ce point de vue identiques aux albumines hétérogènes, que, en un mot, cette réaction dépend encore de l'état dans lequel se trouve l'organisme traité par rapport à l'antigène injecté.

On peut dire, par exemple, qu'un organisme fortement immunisé (activement) pour la toxine tétanique ou diphthérique réagit de la même façon à l'injection d'une nouvelle dose d'une de ces toxines qu'un organisme neuf à l'injection d'une albumine homogène ; ou, en

d'autres termes, que la transformation que subiront la toxine et l'albu-
mine injectées, pour devenir assimilables ou éliminables, s'accomplit
par une réaction de la même nature.

Il est, en effet, peu probable qu'une albumine, même homogène,
injectée dans le sang ou sous la peau, puisse être assimilée sans subir
aucune sorte de transformation, et les troubles anaphyactiques légers
que l'on constate parfois après la transfusion de 100 ou 200 c c de
sang complet : frissons, excitation cérébrale, dyspnée, refroidissement
périphérique, semblent bien prouver que, dans ce cas aussi, il n'y a pas
d'assimilation sans transformation préalable—ou, en d'autres termes,
qu 'il y a dans tout organisme normal un anticorps en excès pour les
albumines homogènes—, et que cet anticorps réagit sur ces albumines
d'une façon analogue à celle de l'antitoxine sur la toxine.

Nous reviendrons plus loin sur cette question, que nous ne pouvons
qu'indiquer ici ; pour le moment, il importe de faire ressortir encore
de ce qui précède que si *la nature des réactions* provoquées par les
antigènes semble être déterminée, selon toute apparence, par les pro-
priétés physico-chimiques de l'état colloïdal de ces substances, *les effets
de ces réactions* sur l'organisme dépendront principalement, sinon
exclusivement, de la nature, des propriétés biologiques et da la quantité
des anticorps-normaux ou en excès.

Et alors, en se plaçant à ce point de vue de leurs effets biologiques,
on peut diviser les réactions entre antigènes et anticorps en deux
grands groupes :

1° Celles qui ont pour effet l'immunité seule et, dans ce cas, se
trouvent les toxines diphtérique, tétanique, peut-être botulinique et
les albumines homogènes.

2° Celles qui ont pour effet l'immunité et l'anaphylaxie, ou seule-
ment l'anaphylaxie, et à ce groupe appartiennent tous les autres anti-
gènes, microbes et albumines hétérogènes.

Et on constate que, *au point de vue physico-chimique :*

1° Les antigènes *exclusivement immunisants* forment avec leurs
anticorps des *composés solubles.*

2° Les antigènes *immunisants et anaphylactisants ou exclusive-
ment anaphylactisants,* forment avec leurs antigènes des *composés
insolubles.*

Dans le premier cas, les *anticorps en excès solubilisent* le colloïde
antigène.

Dans le deuxième cas, *ils le précipitent.*

Et cette différence dans la nature des composés, qui peuvent être solubles ou insolubles, a pour résultat un fait de la plus haute importance au point de vue biologique, ainsi qu'au point de vue de l'évolution des états pathologiques, à savoir:

1° Dans les cas du type diphtérie, quand les anticorps forment avec les antigènes des composés solubles, l'apparition des anticorps en excès coïncide avec la guérison.

2° Dans tous les autres cas où les anticorps forment avec leurs antigènes des composés insolubles, l'apparition des anticorps en excès coïncide toujours avec le commencement de la période d'état de la maladie; ou, en d'autres termes, avec les premiers symptômes pathologiques appréciables.

Jusqu'à présent on n'a pas eu l'idée de considérer les réactions que nous venons d'énumérer dans leur ensemble.

Il y a bien une théorie (Ehrlich) pour expliquer la genèse de l'état pathologique et de la guérison dans les maladies provoquées par les toxines proprement dites (diphtérie et tétanos); on admettait bien, généralement, que dans les autres maladies infectieuses les différents aspects de l'état patholoquique étaient causés par la pullulation de différents microbes et par les exo et endotoxines produites dans l'organisme par ces microbes, sans trop chercher à s'expliquer sur le mécanisme de ces réactions; on a formulé, enfin, depuis Ch. Richet, plusieurs théories pour expliquer la nature et le mécanisme des réactions anaphylactiques sans chercher à connaître la raison d'être intime et la genèse de ces réactions.

Et si les cliniciens signalaient de temps à autre des "syndrômes anaphylactiques" dans quelques maladies infectieuses (Ivanoff dans le paludisme) ou des "crises anaphylactiques" consécutives aux injections de certains médicaments (iodures, antipyrine, arsenobenzènes), les vrais "anaphylactistes" ne voulaient y voir qué analogies superficielles ou accidentelles, parce que dans ces cas, les crises anaphylactiques ne se produisaient pas dans les mêmes conditions que dans leurs expériences.

Or, nous avons vu que les réactions immunisantes et anaphylactiques avec production des anticorps ne peuvent être provoquées que par des colloïdes, parce que les cristalloïdes ne provoquent jamais de réactions analoques; parce qu'enfin une albumine antigène perd ses

propriétés d'antigène en même temps qu'elle aura cessé d'être un colloïde, c'est à dire quand elle se trouvera transformée en molécules d'acides-aminés libres.

La raison d'être de toutes ces réactions doit donc être cherchée dans l'état colloïdal des antigènes, et ce n'est qu'en cherchant à analyser les propriétés physico-chimiques des colloïdes, ainsi que les transformations que ces substances subissent dans le milieu intérieur de l'organisme qu'il sera possible de trouver pourquoi l'organisme est obligé de produire des anticorps en excès, quel est le rôle de ces anticorps et de quelle nature peuvent être les réactions entre les anticorps et les antigènes.

Mais nous n'avons pas encore envisagé tous les éléments du problème à résoudre pour aborder avec fruit les explications définitives, et pour bien poser la question et y répondre en connaissance de cause, nous ne pouvons mieux faire que d'en exposer tous les détails dans un exemple précis.

ÉVOLUTION DE LA FIEVRE TYPHOÏDE

Pathogénie—Période d'incubation.—Le moment de l'infection n'est révélé par aucun symptôme appréciable, mais on peut considérer comme démontré qu'un certain nombre de microbes de la typhoïde, pris par la bouche, résistent à l'action de la digestion gastrique et pénètrent vivants dans l'intestin où ils peuvent se multiplier.

La durée de la période d'incubation est déterminé par la quantité ou la virulence des microbes avalés et par la proportion de microbes restés vivants ou détruits par la digestion gastro-intestinale, et nous sommes obligés d'admettre aussi que les produits de la bactériolyse des microbes détruits (les endotoxines) ne sont pas complètement digérés, c'est à dire transformés en acides-aminés libres, qu'en un mot, une certaine quantité de ces produits reste à l'état colloïdal dans l'intestin et peut être absorbée dans cet état par les cellulules de la muqueuse intestinale et passer dans les capillaires.

Cette première absorption détermine des points de congestion plus ou moins légère, analogue à celle que l'on observe dans le derme quand on injecte de petites quantités de microbes morts sous la peau ; et cette congestion favorise à son tour la pénétration dans la muqueuse et dans les capillaires de nouvelles quantités de produits de la bactériolyse, et même de microbes vivants. L'étendue ainsi que la gravité de la congestion augmentent.

Les premiers microbes qui ont pénétré dans le sang sont certainement phagocytés et portés dans les organes hémopoïétiques, où ils sont

plus ou moins complètement detruits et où ceux qui restent vivants produisent des petits foyers d'infection qui se traduisent par de petites lésions.

Pendant toute cette période, les antigènes typhiques les sécrétions microbiennes ainsi que les produits de la bactériolyse se trouvent *en excès* par rapport aux *anticorps normaux* intracellulaires qui préexistent dans tout organisme sensible à l'action d'un antigène. Les points de congestion sont causés par la *fixation en surcharge*[1] des antigènes par les anticorps qui se trouvent normalement sur place à l'intérieur des cellules qui ont été mises en présence d'un excès d'antigène.

C'est ainsi, par exemple, qu'une injection intra ou hypodermique d'antigène, qui produira une légère congestion locale, ne sera suivie d'aucune réaction appréciable, quand elle sera faite dans la veine, parce que, dans ce dernier cas, l'antigène sera réparti sur un beaucoup plus grand nombre de cellules et ne sera pas en excès.

Mais en même temps, sous l'action continue de nouvelles quantités d'antigène provenant de la mort spontanée ou de la destruction de microbes qui continuent à se multiplier, il se forme dans l'organisme des quantités de plus en plus grandes d'anticorps, et l'excès de cet anticorps, qui *surcharge* d'abord, jusqu'à un certain maximum, les cellules à l'intérieur desquelles il s'est formé, est versé ensuite dans le san par une série de décharges assez brusques.

A un moment donné, l'anticorps apparaît donc *en excès* dans le sang, malgré la pullulation continue des microbes et on doit nécessairement en conclure que la production de l'anticorps est plus rapide que celle del'antigène.

Le moment où on commence à trouver de l'anticorps en excès dans le sang coïncide généralement avec l'apparition des premiers symptômes graves et caractéristiques de la maladie. C'est la fin de la période d'incubation et le commencement de la période d'état.

Si alors, on pouvait se rendre compte de l'état dans lequel se trouve l'organisme infecté, au point de vue de l'immunité et de l'anaphylaxie; si, en un mot, on pouvait arrêter l'évolution de l'infection à la fin de la période d'incubation ou au moment de l'apparition de l'anticorps en excès, on trouverait:

1° Qu'il est devenu plus résistant à une nouvelle infection ou, en d'autres termes, qu'il a acquis un certain degré d'immunité antiinfectieuse active;

2° Qu'il est devenu sensible à l'anaphylaxie active.

[1] J. Danysz.

L'expérience est irréalisable dans ces conditions, mais le résultat serait le même si, au lieu d'une infection par les microbes vivants, l'organisme avait reçu une injection ou une série d'injections de microbes morts.

Au moment de l'apparition des anticorps en excès, il sera immunisé contre une dose de microbes vivants pathogènes pour les témoins, et anaphylactisé pour une dose non pathogène de microbes morts, et *enfin hypersensible pour une dose de microbes vivants supérieure à son degré d'immunité antiinfectieuse.*

Que le microbe soit vivant ou mort, à la fin de la période d'incubation, l'organisme se trouvera exactement dans les mêmes conditions au point de vue de l'immunité et de l'anaphylaxie. Si les suites sont différentes, c'est que le microbe vivant continue à pulluler ; et si on continuait à injecter les microbes morts en doses convenables, à partir du moment où l'organisme est surchargé par les anticorps en excès, on pourrait très probablement, et même certainement, reproduire le tableau de la période d'état d'une infection spontanée.

En effet, la pratique des vaccinations antityphiques a montré que les sujets plus ou moins immunisés par une infection typhoïde antérieurement guerie sont infiniment plus sensibles à la réaction vaccinale que les sujets neufs, et dans les cas d'empoisonnements alimentaires causés par les paratyphiques avalés en doses massives, les crises sont d'autant plus graves que les sujets atteints se trouvent plus fortement immunisés.[2]

Mais alors, pourrait-on se demander, pourquoi une infection spontanée ne s'arrête-t-elle pas à la fin de la période d'incubation puisque l'infecté se trouve à ce moment plus immunisé qu'au début de l'infection ? Cela arrive certainement beaucoup plus souvent qu'on ne l'a supposé jusqu'à présent et, non seulement pour les typhoïdes, mais pour toutes les maladies infectieuses. Dans certains cas, les quelques légers symptômes observés permettent de caractériser la maladie qui "avorte," mais la grande majorité de ces cas échappe à toute observation et l'immunité acquise ne pourrait être révélée que par un sero-diagnostique approprié ou par la réaction des opsonines.

L'explication de ces faits paraît très simple. La continuation de l'évolution de la maladie, à partir de la fin de la période d'incubation, ou son avortement dépend nécessairement du degré de l'immunité

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[2] Une dizaine de personnes, dont une seule surement immunisée pour un paratyphique normalement peu virulent, ont avalé par accident une forte dose de ce paratyphique mélangé à du lait. Toutes ont été plus ou moins indisposées, seule la personne immunisée a été gravement malade. Cette observation peut donc tenir lieu d'une véritable expérience.

acquise ou de la quantité d'anticorps formé, et de la quantité de microbes qui existent à ce moment dans l'organisme (parce qu'on n'est jamais immunisé que contre une certaine dose de microbes) ; c'est donc le rapport entre ces deux quantités qui fera pencher la balance d'un côté ou de l'autre, et les différences individuelles entre les degrés d'immunité naturelle ou acquise. Au moment de l'infection ainsi qu'entre les quantités (ou les virulences) des microbes infectants, expliquent très bien que ces rapports peuvent être très variables.

Dans l'appréciation des résultats de ces réactions il faut encore tenir compte, d'une part, de ce fait prouvé par l'expérience que la quantité de l'anticorps formé à un moment donné, ainsi que la rapidité avec laquelle il se forme, est, dans certaines limites, inversement proportionnelle à la quantité ou à la virulence de l'antigène injecté ; d'autre part, que le résultat final de toutes ces réactions peut dépendre, non seulement de l'action directe de l'antigène sur l'anticorps ou la cellule qui le contient, mais du trouble que la lésion une fois produite, que la "complication" apportera dans le fonctionnement de l'organisme.

Ceci dit, et s'il en est ainsi, comment peut-on se représenter les causes et la genèse des différentes manifestations pathologiques qui caractérisent les maladies infectieuses septicémiques et, en particulier, la fièvre typhoïde ?

Nous avons vu que pour les maladies causées par les toxines (type diphtérie) la guérison commence avec l'apparition des anticorps en excès, tandis que dans le cas de la typhoïde, c'est la maladie qui commence précisément à ce moment. On peut donc affirmer que la période d'incubation de la typhoïde coïncide avec la période d'état ou la maladie proprement dite dans la diphtérie, ou encore, et cela sera plus exact, que, dans ce dernier cas, il y a simultanément incubation au point de vue de la production des anticorps et manifestations pathologiques.

Et s'il en est ainsi, on peut admettre, en se rapportant à ce qui précède, que dans la diphtérie les manifestations pathologiques résultent de l'action directe de l'antigène — toxine sur l'anticorps intracellulaire normal et que les composés de cet antigène avec l'anticorps en excès ne provoquent pas d'état d'anaphylaxie active, tandis que dans la typhoïde, on peut supposer que *l'antigène-sécrétion microbienne* n'est pas pathogène pour les tissus normaux, ou bien que cette sécrétion n'est pas du tout "antigène," et que les symptômes pathologiques qui se manifestent au moment de l'apparition des anticorps en excès, ne peuvent être que le résultat de la combinaison de ces anticorps avec les *antigènes produits de la bactériolyse.*

Le syndrôme de la période d'état dans la typhoïde serait donc exclusivement anaphylactique.

Au point de vue pathogénique, on peut donc résumer les deux cas dans les formules suivantes:

Diphtérie: Toxine + Anticorps normal = maladie, Toxine + Anticorps en excès = guérison.

Typhoïde: T? An = O? Produits de la bactériolyse + Anticorps normal = incubation; Produits de la bacteriolyse + Anticorps en excès = anaphylaxie.

En résumé, nous avons vu que dans la typhoïde il n'y a pas de toxine-sécrétion microbienne-antigène analogue à celle de la diphtérie et s'il y en avait une, alors, ou bien elle serait neutralisée à l'apparition de l'anticorps en excès, à la fin de la période d'incubation (comme dans la diphtérie), et ne pourrait plus produire aucun trouble apparent, ou bien, si elle devenait à ce moment pathogène, elle ne pourrait provoquer que des troubles anaphylactiques.

Mais, dans la typhoïde, il y a certainement pénétration et multiplication des microbes, c'est à dire des produits de la bactériolyse à l'état d'albumines, et il est absolument certain que ce sont ces albumines qui provoquent les troubles caractéristiques de la période d'état de la maladie, comme le ferait toute autre albumine hétérogène par ses combinaisons avec son anticorps en excès.

Donc, on peut conclure avec certitude que, dans la typhoïde, la maladie consiste en une crise d'anaphylaxie chronique ou, en d'autres termes, en une série de crises anaphylactiques successives dont le rythme est déterminé à chaque moment par les trois facteurs suivants:

1° *L'apport de l'albumine-antigène par la multiplication des microbes;*

2° *La quantité d'anticorps en excès formé par l'organisme;*

3° *L'influence des lésions produites dans les différents tissus et organes sur l'état général de l'organisme.*

Toutes les maladies infectieuses septicémiques doivent nécessairement se trouver dans le même cas. Un microbe septicémique ne peut, en effet, être pathogène que si son albumine est "antigène" pour l'organisme, et s'il en est ainsi, il y aura toujours anaphylaxie.

Il est possible, bien entendu, que, dans certains cas, l'action des albumines soit compliquée par celle des sécrétions toxiques, mais l'étude de ces sécrétions à l'état pur, c'est à dire complètement dépourvues des

produits de la bactériolyse, est encore trop incomplète aujourd'hui pour qu'il soit possible de savoir si oui ou non elles peuvent être toxiques et produire des anticorps.

Toutefois, et nous ne saurions trop le répéter, cette question ne peut avoir qu'un intérêt de second ordre dans la pathogénie des troubles observés pendant la période d'état, parce qu'il est évident que, si ces troubles *ne se manifestent que simultanément avec l'apparition dans l'organisme des anticorps en excès, ces troubles ne peuvent être que de nature anaphylactique,* peu importe qu'ils soient produits exclusivement par ces dernièrs et par exotoxines.

ANAPHYLAXIE

L'étude de la pathogénie des maladies infectieuses nous a donc conduit d'une façon irrésistible, par une série de déductions loqiques tirées d'expériences précises à concevoir les manifestations pathologiques de la période d'état comme des troubles de nature exclusivement anaphylactique.

Expliquer la nature, le mécanisme et la raison d'être de l'anaphylaxie équivaudrait donc à connaître la raison d'être de toutes les maladies septicémiques; donner une idée générale et directrice unique à toutes les recherches concernant ces maladies.

Voyons donc ce que nous en savons.

Pour Ch. Richet, à qui revient le mérite de la découverte de l'importance biologique du fait et du mot, l'anaphylaxie était un *état d'hypersensibilité* dans lequel se trouvait un organisme à la suite d'une ou d'une série "d'injections préparantes" d'un antigène, parce que le même antigène, pas ou peu toxique à la première injection, s'est montré très toxique à la seconde, à la "injection déchaînante." Et pour Richet, cette toxicité résultait de la formation d'un poison spécial "apotoxine" formé par la combinaison de l'antigène avec une "toxogénine" hypothétique.

Pour Besredka, il n'y a pas de poison dans l'hypersensibilité anaphylactique. En disant il y a quelques années (1907) "D'une manière générale, la plupart des faits rapportés semblent indiquer que les phénomènes d'anaphylaxie et d'antianaphylaxie se réduisent aux actions de *précipitation* et *d'adsorption* qui régissent les rapports des colloïdes entre eux," ce savant avait, ainsi qu'il le dit dans un ouvrage tout récent,[3] "uniquement pour but d'opposer l'idée d'un processus physique à celle d'un poison chimique déterminé.

Ensuite, après avoir analysé avec sa clarté et son talent habituels les nombreux travaux de Friedberger, Neufeld et Dold, Doerr et Russ, Mutermilch, Bordet, Kraus, Nicolle, Vaughan et Wheeler, etc., et passé au crible de la critique les théories de ces auteurs, tantôt chimiques, tantôt physiques, Besredka complète sa première conception en distant: "Ce qui domine l'anaphylaxie et l'antianaphylaxie, ce n'est pas le poison ni l'antipoison, mais c'est, d'une part, la *rapidité* avec laquelle s'effectue la rencontre du sensibiligène et de la sensibilisine et, d'autre part, le lieu de cette rencontre qui est probablement le système nerveux."

Les causes de la "crise" du "choc anaphylactique" seraient donc purement physiques pour Besredka; toutefois, nous lisons quelques lignes plus haut, dans le même ouvrage (p. 142): "Que se passe-t-il lors de l'injection d'épreuve? L'antigène nouvellement arrivé rencontre la sensibilisine déjà préformée. Leur *affinité* a pour effet de provoquer une réaction intense. Soit que cette réaction rompe l'équilibre de certaines cellules nerveuses, au niveau desquelles a lieu *la combinaison,* soit que celle-ci s'accompagne de dégagement ou d'adsorption d'énergie calorique ou autre, nous assistons à une série de phénomènes, toujours les mêmes, qui constituent le choc anaphylactique."

L'auteur ne s'explique pas sur la nature purement physique ou chimique, ou les deux à la fois, des *affinités* et des *combinaisons* qu'il fait intervenir dans la réaction, mais, avec un peu de bonne volonté, on peut expliquer l'apparente contradiction exprimée dans les deux phrases qui précèdent. Les affinités sont très probablement de nature chimique et les combinaisons aussi; mais, dans la conception de Besredka, le produit formé par la combinaison n'agit pas par ses propriétés chimiques (poison), mais uniquement par ses propriétés physiques (précipité) ou encore, ce n'est pas le produit déjà formé qui provoque les manifestations pathologiques de l'état anaphylactique, mais uniquement la rapidité avec laquelle il se forme et, dans ce cas, à son avis, le précipité ne serait pas nécessaire.

Ainsi, en dernière analyse, qu'il y ait ou non formation du précipité, il résulterait des expériences de Besredka et des interprétations qu'il en donne lui-même, que c'est le facteur *temps* ou la *durée,* c'est à dire une des conditions de la réaction, qui doit nécessairment être chimique, ou plutôt physico-chimique, puisqu'il s'agit de colloïdes, qui serait *la cause unique du choc anaphylactique.*

F. G. Novy et P. H. DeKruif[4] reviennent encore, dans un ouvrage considérable à l'idée d'une anaphyloxotine ou "taraxine" soluble formée

[3] Anaphylaxie-Antianaphylaxie, Masson & Cie, Ed. Paris, 1917.
[4] Jour. Am. Med. Assn., 1917, 68, p. 1524; Jour. Infect. Dis., 1917, 20, pp. 499-833.

dans l'organisme par une substance "taraxigène." Le choc anaphylactique serait le résultat d'une sorte de réarrangement intramoléculaire tautométrique de certaines substances très labiles contenues dans le sang.

Au fond, il est évident que toutes ces appréciations différentes ne sont que des querelles de mots.

On a discuté et on discute encore, dans la littérature médicale courante, au sujet de différents phénomènes et processus concernant l'immunité et l'anaphylaxie, à peu près de la même façon, que nos pères et grand'pères discutaient sur les symptômes, la pathogénie et l'évolution des maladies infectieuses avant la découverte des microbes et on a créé, pour chercher à expliquer ces choses, une terminologie compliquée et barbare quie a, en outre, le grand inconvénient de donner des illusions trompeuses d'une précision qui n'existe pas.

La confusion, les malentendus ne proviennent, dans l'immense majorité des cas, que de l'imprécision forcée de termes qu'on a été obligé d'employer pour essayer de caractériser les réactions entre substance dont on n'envisageait que quelques propriétés biologiques.

Et les différences dans la nature des réactions du choc anaphylactique et de l'anaphylaxie chronique (phénomène d'Arthus) que l'on a voulu établir dans l'anaphylaxie du cobaye, du lapin, de la chèvre, du cheval ou du rat et même des cobayes de France et d'Amérique, ne peuvent provenir que de ce fait encore, qu'on n'avait, jusqu'à présent, aucune idée générale pour se guider dans l'interprétation des phénomènes cliniques observés et des expériences.

Or, peu importent les mots *d'anaphylaxie* ou de *taraxie, d'apo* ou *d'anaphylatoxines* ou de *taraxines* et les significations qu'on a voulu leur donner en limitant les phénomènes tantôt au "choc," tantôt à la crise d'une durée plus ou moins longue, tantôt encore à des accidents tardifs de différentes natures en prenant pour caractère distinctif tantôt la pathogénie, tantôt les symptômes.

Pour définir un phénomène, il ne suffit pas d'indiquer le procédé par lequel on peut le provoquer; on doit se préoccuper de savoir en quoi il consiste, c'est à dire déterminer les symptômes par lesquels il se manifeste, les causes qui peuvent provoquer ces symptômes et, avant tout, les propriétés des éléments qui concourent à provoquer ces symptômes.

Or, si nous ne connaissons pas toutes les propriétés de tous les antigènes et de tous les anticorps, ainsi que de tous leurs composés, nous pouvons affirmer avec certitude que l'état anaphylactique ne peut se produire que par les antigènes qui forment avec leurs anticorps des composés insolubles. Nous avons vu qu'il n'y a pas d'anaphylaxie *malgré la rapidité des réactions* pour les toxines (diphtérique et tétanique) qui forment avec leurs antitoxines des composés solubles, et qu'il y a toujours anaphylaxie pour les albumines, les microbes et leurs bouillons de culture qui provoquent la formation des précipitines.

La théorie la plus commode, et qui expliquerait le mieux l'ensemble des faits connus actuellement, consisterait donc à dire, que la crise anaphylactique est une réaction brusque de coagulation provoquée par la rencontre dans l'organisme d'une certaine dose d'antigène avec une certaine dose d'anticorps. Peu importe que l'anticorps préexistât dans l'organisme ou qu'il se soit formé à la suite d'une préparation spéciale.

Dans le cas de vrais antigènes, ce sont ces derniers qui sont coagulés ou précipités par une substance de l'organisme (anticorps) ; dans les cas où la crise est provoquée par la première injection de substances non antigènes, telles que l'iodoforme, l'antipyrine, etc., c'est la substance injectée qui provoque la coagulation d'une substance de l'organisme. La nature des réactions sera toujours la même, et, bien que ces derniers cas n'entrent pas dans les cadres de cette étude, il n'est pas sans intérêt d'en tenir compte pour éviter les confusions et les malentendus possibles.

Les effets de ces réactions coagulantes seront différents suivant que le précipité se formera exclusivement dans le sang ou en même temps dans le sang et dans un certain nombre de cellules, ou encore exclusivement dans les cellules et dans ces derniers deux cas, suivant l'importance de l'anticorps intracellulaire dans la vie de la cellule, ainsi que de l'importance du rôle de la cellule dans la vie de l'organisme.

Il serait superflu d'insister ici sur la symptômatologie de l'anaphylaxie à ses différents degrés et dans ses différentes localisations, que l'on trouvera aujourd'hui dans tous les traités de pathologie ; mais il importe de faire remarquer que dans l'ensemble des symptômes qui caractérisent une crise anaphylactique, il y a tout à la fois :

et l'action d'un poison nouvellement formé dans l'acception imprécise courante de ce mot (Richet, Vaughan et Wheeler, Friedberger, etc.) ;

et réaction chimique (combinaison de l'antigène et de l'anticorps) ;

et réaction physique ou adsorption (Mutermilch) ;

et action mécanique du précipité : embolies et infractus ;

et fonction du temps ou la durée de la réaction (Besredka) ;

et intervention des leucocytes dans le transport et la transformation des précipités dans les organes hémopoïétiques ;

et enfin, dominant le tout, le mécanisme comme les effets de toutes ces réactions, intervention du système nerveux central.

Suivant le point de vue auquel s'est placé l'expérimentateur, c'est tantôt l'un, tantôt l'autre de ces agents qui prédominait dans son esprit et déterminait de préférence telle ou telle autre interprétation du fait. Chacune de ces théories contenait une partie de vérité, mais ce n'était pas toute la vérité, et cela ne pouvait pas être autrement tant qu'on se bornait à considérer séparément les effets apparents des réactions sans chercher à pénétrer leur raison d'être et leur mécanisme intime.

Or, nous avons vu :

1° Que seuls les antigènes peuvent préparer l'organisme à l'état anaphylactique ;

2° Que tous les antigènes sont des colloïdes ;

3° Que seuls les colloïdes sont antigènes.

Que, par conséquent, la raison d'être de la formation des anticorps doit être cherchée dans l'état colloïdal des antigènes ou, en d'autres termes, que c'est en cherchant à nous rendre compte des propriétés physico-chimiques et biologiques des colloïdes et en étudiant les transformations que les colloïdes hétéogènes et homogènes subissent dans le milieu intérieur de l'organisme que l'on parviendra à apprendre·

Pourquoi les colloïdes provoquent la formation des anticorps;

Pourquoi et dans quelles conditions les colloïdes forment avec les anticorps normaux ou en excès des composés solubles ou insolubles;

Pourquoi les composés d'antigènes avec leurs anticorps en excès sont inoffensifs quand ils sont solubles, et pathogènes quand ils sont insolubles; et pourquoi cela doit être nécessairment ainsi.

LES COLLOIDES

On sait que les colloïdes sont des substances qui ne cristallisent pas, que l'on ne peut, par conséquent, pas isoler de leur milieu à l'état pur, et on sait aussi qu'en cherchant à purifier un colloïde, on arrive généralement à le faire cesser d'être un colloïde.

Suivant l'expression si heureuse de E. Duclaux, en cherchant à analyser une albumine par des méthodes employées en chimie, on fait comme si on analysait une montre enfermée dans un sac. On y trouverait du fer, du cuivre, de l'argent, de l'or, les éléments qui composent le verre, mais on ne saurait jamais conclure de cette analyse que ces éléments arrangés d'une certaine façon et mis en mouvement suivant un certain état d'équilibre, constitueront un ensemble qui nous permettra de mesurer le temps.

On peut dire qu'en analysant une montre de cette façon, après l'avoir broyée dans un mortier pour rendre plus faciles les attaques par les acides forts, ou après l'avoir fait fondre à une température voisine de 1000°, on détruit, non seulement l'instrument qui marque l'heure, mais encore tous les rouages qui auraient pu nous permettre de reconnaître le rôle individuel de chacun d'eux dans le fonctionnement de l'ensemble qui aboutit au mouvement des aiguilles.

Mais depuis on a appris à mieux faire. En soumettant une albumine à une action moins brutale, à celle de la digestion gastro-intestinale, par exemple, on peut la dégrader peu à peu sans détruire les entités complexes "les rouages" qui la composent, et on a constaté ainsi qu'en passant par toute une série de dégradations successives, toute albumine qui est un colloïde finit par être scindée en acides-aminés qui sont des cristalloïdes.

Nous savons que les molécules d'un sel en solution se collent les unes aux autres pour former des cristaux quand il n'y a plus assez de solvant pour les tenir écartées à une certaine distance; mais nous ne savons pas encore comment et pourquoi les acides-aminés, qui peuvent exister à l'état de molécules libres et qui obéissent alors aux lois régissant les solutions salines, s'agrègent en éléments complexes pour former un colloïde.

Il serait surtout intéressant de savoir sous l'action de quel agent physique ou chimique (électricité, magnétisme, affinité, etc.) ces molécules d'acides-aminés sont reliées et retenues ensemble. On le saura certainement un jour : Pour le moment, il faut nous borner à l'étude des propriétés biologiques et physico-chimiques de l'albumine telle que nous la trouvons dans son milieu complexe et des substances qui résultent de ses dégradations successives.

Bien qu'incomplète, cette étude nous donne déjà une foule de renseignements intéressants et suffisamment précis pour le but que nous poursuivons : apprendre comment et pourquoi un organisme peut

devenir malade et, peut-être aussi, comment il peut guérir dans les meilleures conditions.

On a donc constaté que de tous les produits de dégradation d'une albumine, ceux-là seuls sont antigènes qui sont encore à l'état de colloïdes. Les acides-aminés libres ne sont plus antigènes.

C'est donc tout d'abord une confirmation du fait que nous avons relevé plus haut que seuls les colloïdes peuvent être antigènes, et il est possible d'en conclure de suite ceci : que les albumines étrangères prises sous forme d'aliments que la digestion gastro-intestinale sera incapable de digérer, c'est à dire de transformer en acides-aminés et qui pourront passer dans le milieu intérieur de l'organisme à l'état de colloïdes, provoqueront la formation des anticorps et, par conséquent, l'état anaphylactique.

Ce sont là les causes des intolérances anaphylactiques habituelles ou accidentelles pour certains aliments, des infections par voie buccale, individuelles ou spécifiques dans les typhoïdes, le choléra, la tuberculose, etc.

L'organisme ne peut se nourrir d'albumines étrangères qu'à la condition *de les assimiler,* c'est à dire les transformer en albumines de son espèce, et on sait que pour ce faire, il ne peut less prendre qu'à l'état complètement dégradé ou transformées en acides-aminés, et tel est généralement le rôle de la digestion gastro-intestinale.

Que deviennent alors les albumines incomplètement digérées, qui ont pénétré dans le milieu intérieur de l'organisme à l'état de colloïdes ?

Elles ne peuvent être ni assimilées, ni éliminées à l'état de colloïdes et alors deux hypothèses sont possibles ;

ou bien, elles s'accumuleront quelque part, comme le ferait tout autre corps étranger, inassimilable, et seront séquestrées par les leucocytes ;

ou bien, c'est le milieu intérieur de l'organisme qui parachèvera la digestion gastro-intestinale incomplète et les rendra assimilables et éliminables sous forme d'acides-aminés.

C'est cette dernière hypothèse qui se réalise dans tous les cas connus.

Il est vrai de dire que, jusqu'à présent, nous n'avons jamais encore assisté à cette digestion ; mais si nous n'en avons pas de preuves expérimentales directes, nous savons avec certitude par des expériences nombreuses (Hambourger et Moreau, etc.) que, quand on injecte par exemple à un lapin du sérum de cheval, on retrouve ce sérum dans le sang du lapin plus ou moins longtemps après l'injection, suivant la dose

injectée, mais que ce sérum finit toujours par disparaître à un moment donné et que cette disparition, assez brusque, coïncide toujours avec l'apparition, dans le sang du lapin, de l'anticorps spécifique.

Or, au point de vue de la réaction qui s'en suit, il importe peu par quelle voie (intestinale, sous-cutanée ou intraveineuse) le colloïde antigéne a pénétré dans l'organisme.

La transformation dans l'organisme du colloïde en sel n'a pu être suivie jusqu'à présent avec quelque précision que pour un colloïde obtenu par synthèse et que des recherches toutes récentes permettent de considérer comme un antigéne.

C'est le disodo dioxydiaminoarsenobenzène stibiobromo argentique. (ou produit 102).

A en juger par les expériences et les analyses de Mlle Michel, ce produit injecté dans le sang des lapins à l'état de colloïde est éliminé en entier par les reins et l'intestin à l'état de cristalloïde. On peut donc affirmer que, dans ce cas, le colloïde a subi dans l'organisme la transformation que l'on appelle communément une digestion complète, et puisque ce colloïde possède toutes les propriétés biologiques et physico-chimiques communes à tous les antigénes, quelle qu'en soit la nature et l'origine, puisqu'il provoque dans l'organisme la même série de réactions dans les mêmes conditions, on peut bien admettre aussi, en retour, que tous les autres antigénes subiront dans l'organisme des transformations de même nature, qu'introduits à l'état de colloïdes, ils seront digérés, c'est à dire d'abord transformés en sels et alors seulement assimilés ou éliminés.

Par conséquent, en nous plaçant tout d'abord à un point de vue purement biologique, et en mettant à contribution tout le matériel expérimental connu, on est obligé d'admettre que l'introduction dans le milieu intérieur de l'organisme d'un colloïde-antigéne *digestible* provoquera toujours une réaction de digestion de la part de l'organisme, tout comme l'introduction d'une albumine dans l'appareil digestif. Et on peut ajouter avec autant de certitude que la formation de l'anticorps en excés qui apparaît dans le sang à la fin de la période d'incubation ne peut être que le résultat d'une réaction normale et commune à toute cette cellule vivante qui cherchera toujours, tant qu'elle vivra, à reproduire et à multiplier une substance dont elle a besoin et qu'elle aura perdue à la suite d'une combinaison neutralisante avec une substance étrangère (Ehrlich).

On peut se représenter facilement la marche de ce processus de la façon suivante :

Tout organisme possède pour toute albumine *digestible* une certaine affinité normale (et il n'y a là rien qui puisse surprendre puisque toutes les albumines sont construites sur le même plan et appartiennent à la même famille chimique) ou, en d'autres termes, une certaine *dose* d'affinité normale pour une certaine dose d'albumine étrangère.

Si la dose d'albumine injectée est strictement équivalente ou inférieure à la dose d'affinité normale, tout l'antigène injecté sera fixé par l'anticorps normal, digéré et assimilé ou éliminé, et l'organisme reproduira et multipliera cet affinité-anticorps normal qui deviendra l'anticorps en excès. Si la dose d'albumine injectée est supérieure à la dose d'affinité normale, l'antigène se fixera sur l'anticorps normal "en surcharge" et l'organisme peut en souffrir, ou bien l'antigène en excès circulera dans le sang jusqu'au moment où l'organisme aura produit une quantité d'anticorps suffisante pour fixer et digérer cet excès d'antigène; et c'est alors seulement, après la disparition totale d'antigène, qu'apparaîtra l'anticorps en excès.

C'est ainsi que s'explique tout naturellement un fait qui a semblé inexplicable jusqu'à présent, à savoir, que si l'injection d'une pétite quantité d'antigène fait apparaître l'excès d'anticorps et l'état anaphylactique en 10 à 15 jours, l'injection d'une grande quantité du même antigène, à un animal de la même espèce ne produira le même effet qu' après un temps d'incubation de quelques semaines ou même de quelques mois, tout simplement parce que l'existence d'un excès d'antigène dans la circulation exclut la possibilité de l'existence simultanée d'un excès d'anticorps dans l'organisme.

Dans quel état se trouvera alors un organisme anaphylactisé?

Il sera surchargé d'anticorps, ce qui veut dire que toutes les cellules qui possédaient une affinité spéciale pour l'antigène injecté auront multiplié la substance qui a fixé l'antigène, en garderont une certaine quantité en surcharge et laisseront passer le reste dans le sang. Il y aura donc un excès d'anticorps dans les cellules et dans le sang. Si, à ce moment, on injecte une nouvelle quantité du même antigène, on provoquera naturellement une réaction de la même nature que la première fois, mais les effets de cette réaction sur l'organisme seront différents, parce que les quantités et les proportions des deux produits ne seront plus les mêmes et aussi, parce que la réaction se passera, non seulement dans les cellules, mais aussi dans le sang.

Nous avons vu plus haut que les composés des antigènes avec leurs anticorps peuvent être solubles, et alors ils sont neutres pour l'organisme (diphtérie, tétanos) ou bien ils forment des coagulums ou des précipités et alors ils sont pathogènes.

Et dans ce dernier cas, comme il y a de l'anticorps en excès dans le sang et dans les cellules, il y aura des réactions pathogènes intravasculaires et intracellulaires.

Ces réactions seront pathogènes, non point parce qu'il se formera à la 2° injection un corps toxique nouveau différent de celui qui s'est formé à la première injection, mais uniquement parce que ce corps nouveau se formera beaucoup plus rapidement et en quantité beaucoup plus grande que la première fois. Les précipités formés dans le sang auront pour résultat des embolies, infarctus, etc., d'où attaques apoplectiques, syncopes, congestions intestinales, oedèmes pulmonaires ou dermiques, etc...., accompagnés d'hypothermie et des lésions intracellulaires qui se traduiront par des symptômes variés et d'autant plus graves que les cellules atteintes joueront un rôle plus important dans l'économie générale. Ces dernières réactions, surtout quand elles intéressent plus ou moins les cellules nerveuses, sont accompagnées d' hyperthermie.

Notons, en passant, que les réactions intravasculaires, quand elles ne seront pas rapidement mortelles, auront toujours des suites bien moins dangereuses que les réactions intracellulaires.

On peut donc affirmer que la raison d'être de la production des anticorps en excès est l'obligation dans laquelle se trouve l'organisme de digérer les colloïdes pour pouvoir les assimiler ou les éliminer, et nous savons aussi que cette digestion provoque des manifestations pathologiques quand le composé d'antigène avec l'anticorps donne un précipité, qu'elle est, au contraire, complètement neutre quand ce composé est soluble.

Il reste à expliquer la raison de cette différence, c'est à dire le mécanisme intime des réactions qui aboutissent d'une part à la formation d'une part à la formation d'un coagulum ou d'un précipité ; d'autre part, à la formation d'un produit qui reste soluble.

Pour répondre à cette question, on ne dispose malheureusement que d'un matériel expérimental très restreint : elle relève, en effet, de la constitution physico-chimique des colloïdes qui est encore très peu connue. Voyons toujours ce que nous en savons et quelles conclusions nous pouvons en tirer au point de vue qui nous préoccupe.

Au point de vue physique, on peut représenter un colloïde comme formé par des granules : *les micelles,* composés d'un nombre plus ou moins grand de molécules d'une seule ou d'un nombre plus ou moins grand de substances différentes.

On ne connaît pas la structure de ces micelles, mais on peut admettre que, quand elles se trouvent en suspension dans un liquide, elles doivent avoir une forme sphérique ou ovoïde, à cause de la tension superficielle.

Le volume des granules du colloïde antigène le plus simple et le plus homogène que nous connaissions, d'un dioxydiaminoarsenobenzène, est variable, non seulement parce que les micelles peuvent contenir un nombre plus ou moins grand de molécules, mais parce que plusieurs micelles peuvent être agrégées en un seul granule.

L'expérience montre, en outre, que dans tout liquide contenant un colloïde en suspension, il y a aussi un certain nombre de molécules libres.

Ainsi, quand on laisse en repos une solution de luargol à 1 p. 400 dans l'eau salée à 5, 6 ou 7 pour 1,000, on constate qu'au bout de 24 heures, il s'est formé dans le liquide qui était, au début, uniformément coloré et limpide, quatre couches superposées, bien distinctes. En haut, une couche liquide limpide peu colorée ; au-dessous, deux couches plus foncées et troubles ; au fond, un précipité floconneux translucide. Au bout de 2 à 3 jours, il n'y aura plus dans le tube qu'un dépôt et un liquide parfaitement transparent et peu coloré.

Ce liquide ne se trouble plus, même quand il est saturé de sels et traverse les membranes dialysantes. Il n'y a donc plus de colloïde, et la façon dont s'est formé et déposé le précipité dans le reste du liquide primitif montre bien qu'il y avait là des granules de grosseures différentes.

Dans une solution exactement disodique, il y aurait environ, après 24 heures, 50 p. 100 du produit dans le précipité et respectivement 25, 20 et 5 p. 100 dans les couches suivantes.

Les proportions d'acides-aminés à l'état de molécules libres et agrégées en granules plus ou moins volumineux peuvent être différentes pour les divers colloïdes et pour le même colloïde suivant les conditions de milieu dans lequel il se trouve ; mais, à en juger par l'allure des réactions entre colloïdes et sels, ainsi que par les résultats que l'on obtient en soumettant les colloïdes à la dialyse, on peut affirmer que dans un sérum, comme dans le blanc d'oeuf, dans une albumine microbienne comme dans une toxine, il y a des granules de volumes

bien différents et des acides-aminés à l'état de molécules libres, comme on l'a constaté pour les arsenobenzènes.

Ainsi, tout en reconnaissant qu'il y a des différences bien tranchées entre la formation d'un cristal dans lequel molécules du sel sont *mécaniquement collées* les unes aux autres en un granule colloïde dans lequel les molécules sont très probablement *rellées par une affinité,* il faut reconnaître aussi qu'il n'y a pas de cloisons étanches entre ces deux sortes de substances, quand on les considère au point de vue de leur propriété physique de traverser les membranes dialysantes.

Il n'y a pas de membrane qui ne puisse être traversée par les gran-ulse les plus petits de tout colloïde. Et il est très important d'en tenir compte, parce que ce sont ces granules les plus petits des antigènes qui traversent les membranes des cellules et qui "amorcent" les réactions en provoquant un état de congestion ou dilatation de la membrane cellulaire, qui devient ainsi perméable pour des granules plus volumineux.

Au point de vue chimique, on sait que chaque molécule de chlorhy-drate de dioxydiaminoarsenobenzène peut se combiner théoriquement avec deux molécules de chlorure d'argent, mais, quand on verse goutte à goutte une solution étendue de chlorure d'argent dans du cyanure de potassium, dans une solution étendue (1 pour 500) d'arenobenzène, on constate que chaque goutte de chlorure d'argent est d'abord précipité en masse sous forme de globule qui se redissout ensuite si l'on a soin d'agiter le liquide, et que pour chacune des gouttes qui se suivent, la dissolution devient plus difficile. Quand on arrive à la proportion d'un peu plus d'une molécule de chlorure d'argent pour une molécule d'arsenobenzène, la dissolution ne se fait plus. On obtient aussi un précipité insoluble dans son milieu en versant brusquement une molé-cule d'une solution concentrée de cyanure d'argent dans 20 molécules d'arsenobenzène, malgré qu' il y a encore dans le liquide assez de ce dernier pour fixer et maintenir en solution 19 autres molécules de cyanure d'argent, et on ne peut s'expliquer la différence de ces résultats qu'en admettant que, dans le premier cas, en opérant lentement dans des milieux étendus et en agitant le mélange, le chlorure d'argent se répartit plus ou moins uniformément entre tous les granules d'arseno-benzène, tandis que dans l'autre cas, chaque molécule de l'arsenoben-zène qui vient en contact avec le chlorure d'argent en fixe deux molé-cules et il se forme ainsi un composé insoluble.

Le granule d'un arsenobenzène peut donc fixer un sel, pour lequel il y a une certaine affinité, en quantité égale à la somme des affinités des molécules qui le composent; mais il est évident qu'il peut aussi en fixer moins, et c'est ainsi que l'on peut expliquer le *phénomène de surcharge* ou de *moindre saturation* que l'on a constaté en étudiant les propriétés des mélanges des antigènes avec leurs anicorps *in vitro* et *in vivo*. Pour les arsenobenzènes, on connait exactement les équivalents chimiques, pour les antigènes biologiques, on ne les moins bien, mais la similitude et même l'identité des allures de toutes les réactions, entre les arsenobenzènes et tous les autres antigènes nous autorisent à admettre qu'il y a aussi identité de constitution physico-chimique.

Les phénomènes d'adsorption et d'hydrolyse, l'action précipitante des sels neutres et dissolvante des milieux acides ou alcalins qui caractérisent tous les colloïdes, nous permettent de considérer chaque granule colloïdal, comme une sorte de cellule pourvue d'une sorte de membrane et que cette cellule peut être hydratée et gonflée, déshydatée et rétrécie; qu'elle peut adsorber, retenir et excréter toutes sortes de sels par le phénomène purement physique d'osmose, sans préjudice des combinaisons chimiques que peuvent contracter ses acides-aminés et autres molécules grâce à leurs affinités.

L'allure des réactions entre colloïdes ou entre colloïdes et sels, ainsi que *la quantité* des substances qui peuvent être combinées ou adsorbées, sont déterminées par les caractères physiques des colloïdes, par la forme et le volume des granules qui le composent et aussi par la proportion des granules de différentes grosseurs; tandis que *"le choix"* des substances avec lesquelles un colloïde peut se combiner et la nature des composés formés dépendra évidemment des affinités chimiques des molécules qui entrent dans la composition des granules.

Il est très possible de se représenter, en effet, que dans un granule composé par exemple de 100 molécules, 10, 20 ou 60 de ces molécules formeront des combinaisons nouvelles, tandis que les autres resteront intactes, et que pour chacune de ces proportions, les propriétés, physico-chimiques, ainsi que les effets biologiques du colloïdes, seront différentes.

Un arsenobenzène est le colloïde-antigène .le plus simple que nous connaissions. Les granules sont de volumes différents et variables, mais tous sont formés de molécules de la même amine. Les antigènes biologiques sont, en général, d'une composition beaucoup plus com-

plexe. La caséine, par exemple, est formée de douze acides-aminés différents (analine, leucine, serine, acide glutamique, acide aspartique, arginine, lysine, hystidine, cystine, tyrosine, phénylalanine, tryptophane), et il est certain qu'entre une caséine et un arsenobenzène on trouvera tous les intermédiaires.

Il serait aussi très intéressant de savoir si toutes les différentes acides-aminés que l'on trouve dans une caséine, un sérum ou un antigène microbien sont réunies en proportions variables dans chaque granule, ou si chacune d'elles forme des granules différents. Ce sont là des problèmes que la chimie biologique parviendra probablement à débrouiller un jour et dont nous ne pouvons pas nous occuper pour le moment. Ce qui paraît certain toutefois, c'est que tous les colloïdes d'une albumine d'oeuf, de sérum ou d'un corps microbien ne deviennent pas "antigènes" quand on les introduit dans le milieu intérieur de l'organisme, ou, en d'autres termes, que ce ne sont pas tous les granules colloïdaux de composition différente d'une albumine qui prennent part au même titre à la formation d'anticorps spécifiques, parce que les anticorps produits par le même antigène chez des animaux de différentes espèces ne sont pas identiques, ainsi que cela résulte des travaux de Levaditi et de Mutermilch[5] sur la production des anticorps anti-Nagana d'une part chez le cobaye, le lapin, et le rat, d'autre part chez la poule.

Les anticorps différents n'ont pu être produits que par des antigènes différents, ce qui veut dire que, dans le cas particulier mis en lumière par les intéressants travaux de Levaditi et Mutermilch, les produits de la bactériolyse du trypanosome ne forment pas un antigène uniforme, mais contiennent un mélange de granules colloïdaux dont la composition et les affinités sont différentes et que, par conséquent, les granules colloïdaux qui sont, antigènes pour l'organisme du lapin, du cobaye et du rat, d'une part; de la poule, d'autre part, ne sont pas composés des mêmes acides-aminés.

Nous avons vu aussi que dans le *Bacillus typhi murium*, la substance virulente pour les souris n'est pas la même que celle qui confère à ce microbe sa virulence pour les rats, et ces deux exemples nous permettent de conclure que dans un colloïde complexe formé de plusieurs sortes d'acides-aminés, ces dernières ne sont pas uniformément réparties entre tous les granules, mais constituent des groupes de granules d'une composition chimique différente.

[5] Levaditi et Mutermilch: Anticorps et espèces animales, Annales de l'Institut Pasteur, 1913, 27, p. 924.

Et s'il en est ainsi, on doit nécessairement en conclure que tous les autres granules colloïdaux d'une albumine ou d'un colloïde complexe *qui ne sont pas antigènes, doivent être forcément digérés, c'est à dire transformés en cristalloïdes* d'une autre façon que les colloïdes antigènes.

Ceci nous amène à aborder la question de la digestion des albumines homogènes qui, à l'exception des cristalloïdes, peuvent être assimilées sans provoquer la formation d'anticorps et sans jamais donner naissance à l'état d'intolérance anaphylactique.[6]

Or, comment peut-on se représenter cette assimilation des albumines homogènes.

Il est impossible d'imaginer la pénétration dans une cellule d'une albumine non dégradée; par conséquent, une albumine homogène doit être transformée en acide-aminés libres tout comme une albumine hétérogène; seulement, cette transformation doit s'opérer d'une autre façon.

A en juger d'après ce que nous savons, les choses se passent comme si une albumine homogène était transformée directement en acides-aminés libres, dans le sang et les humeurs de l'organisme, sans passer par la phase de coagulation; ou, en d'autres termes, comme s'il y avait dans chaque organisme une substance capable de détruire ou de bloquer les liaisons par lesquelles les acides-aminés sont réunis en granules et, en les libérant ainsi, de les rendre assimilables . Et l'exemple de la transformation d'un arsenobenzène colloïde en "novo-arsenobenzène" qui est un sel, par la fixation du formaldéhyde sulfo-xylate de sodium sur le groupement aminé du dioxydiaminoarsenobenzène, nous permet d'affirmer que les choses peuvent, en effet, se passer ainsi.

Il est bien probable que les colloïdes des albumines étrangères qui ne sont pas antigènes sont transformés en cristalloïdes de la même façon que les albumines homogènes, et il n'y a rien là qui doive surprendre quand on songe à l'unité d'origine de toutes les espèces animales et à l'homogénéité de composition élémentaire de toutes les albumines qui diffèrent entre elles, bien plus par les quantités et l'arrangement, que par les qualités des espèces chimiques qui les composent. Ce qui doit différer surtout, ce sont les *liaisons* par lesquelles les molécules-aminés sont réunies en granules colloïdaux.

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[6] Toutefois il est à remarquer que si on injectait à un animal p. ex. de l'extrait de muscle d'un animal de la même espèce, on ne trouverait dans le sang du premier de l'anticorps en excès qu'après la disparition totale du tissu musculaire de l'animal injecté, et on ne sait pas quel serait le résultat d'une expérience bien faite.

Quoi qu'il en soit de ces différences de détail entre les différentes albumines et les colloïdes qui les composent, l'ensemble de nos connaissances sur les conditions de digestion des colloïdes non antigènes et des colloïdes antigènes dans le milieu intérieur de l'organisme nous permettent d'envisager le mécanisme de ces deux sortes de processus de la façon suivante :

Tout organisme doit toujours posséder dans ses humeurs certaines substances "anticorps normaux" *en quantité suffisante* pour transformer rapidement et par une seule opération des quantités quelconques de *colloïdes non antigènes,* en solutions cristalloïdes assimilables ou éliminables et il le fait par le même procédé qui lui permet de digérer ses propres tissus.

Tout organisme peut produire, à la suite d'une préparation convenable, une substance anticorps spécifique pour transformer en cristalloïde chaque *colloïde antigène,* mais, dans ce cas, le processus de cette transformation peut s'opérer de deux façons différentes : 1° en une seule réaction, comme pour les colloïdes non antigènes (toxines-antitoxines) ; 2° par deux réactions successives, dont la première consiste dans la formation d'un coagulum et la deuxième dans la dissolution de ce coagulum (toutes les autres albumines et colloïdes étrangers antigènes).

A en juger par ce que nous savons des transformations des arsenobenzènes, on peut admettre que, dans le cas des colloïdes non antigènes, ainsi que dans le cas des toxines, l'anticorps normal ou en excès agit d'emblée sur les liaisons qui réunissent les acides-aminés en granules colloïdaux et neutralise ces liaisons, tandis que dans le cas de colloïdes-antigènes, les anticorps en excès forment d'abord avec les molécules de ces antigènes d'autres combinaisons qui relient les granules entre eux et provoquent ainsi la formation des précipités ou des coagulums qui rendent la libération subséquente des molécules plus lente et plus difficile.

ANTICORPS

Il nous reste à rechercher quelle peut être la nature physico-chimique des anticorps ; et nous devons reconnaître que c'est là l'élément du problème le moins connu.

Nous savons que la production de l'anticorps en excès est le résultat d'une réaction vitale de la cellule, parce que, si une cellule ou un tissu mort peut fixer une certaine quantité d'un antigène, de la même façon et par la même affinité qu'un tissu vivant, il sera impuissant à repro-

duire et à multiplier cette substance fixatrice qui devient, dans l'organisme vivant, l'anticorps en excès.

Et c'est ainsi que l'on peut expliquer pourquoi la production des anticorps sera nécessairement toujours inversement proportionelle à l'action pathogène des antigènes.

Nous savons aussi que la substance à qui échoit le rôle d'anticorps normal peut remplir différentes fonctions plus ou moins importantes pour la vie normale de la cellule. Cela peut être une substance-organe de sensibilité, ou d'une fonction de nutrition, ou de reproduction, ou enfin une substance de réserve, et dans chacun de ces cas, l'excitation immunisante ou la lésion se traduira par des effets différents sur le fonctionnement du tissu intéressé et de l'économie générale.

On sait encore que ce ne sont pas toujours les mêmes cellules du même tissu qui sont sensibles à l'action des différents antigènes ; dans le cas où ce sont les cellules de système nerveux qui sont exclusivement, ou plus particulièrement sensibles à l'action d'un antigène, la production des anticorps est très précaire, sinon nulle, probablement parce que l'excitation, même très légère de ce tissu, a toujours pour conséquence un trouble profond de l'économie générale. On peut en conclure que, moins important est le rôle du tissu intéressé, plus facile et moins nocif sera le processus d'immunisation.

Voilà à peu près tout ce que nous pouvons dire sur l'origine biologique des anticorps.

Quant à leur nature physico-chimique, nous savons que c'est un des composants des sérums "anti" dont il a été impossible jusqu'à présent de les isoler par dialyse. On serait donc tenté de croire que c'est un colloïde, mais on n'en a aucune preuve directe.

L'exemple de ces transformations des arsenobenzènes qui sont coagulés quand on enlève aux molécules l'acide ou la base qui tiennent les granules colloïdaux en suspension dans l'eau et la redissolution du coagulum ainsi que la dislocation des granules colloïdaux par une sorte de sulfonation, tendrait à prouver que les anticorps pourraient être quelque chose de beaucoup plus simple qu'un colloïde ; mais s'il peut en être ainsi pour les arsenobenzènes, cela semble encore aujourd'hui trop simple pour les anticorps de tous les autres antigènes.

Contentons-nous donc, pour le moment, de garder dans l'esprit la simplicité des réactions qui déterminent les transformations des arsenobenzènes et d'espérer que des recherches prochaines nour feront connaître la nature et le mécanisme des transformations des antigènes biologiques.

RÉSUMÉ

1° Les sels introduits dans le milieu intérieur de l'organisme peuvent être assimilés ou éliminés sans avoir subi aucune transformation, parce qu'ils traversent les membranes dialysantes ;

2° Les colloïdes ne peuvent être assimilés et éliminés dans les mêmes conditions, parce qu'ils ne peuvent traverser les membranes dialysantes ;

3° La digestion gastro-intestinale a pour effet de transformer les colloïdes-albumines spécifiques en sels (acides-aminés) qui ne sont plus spécifiques et avec lesquels l'organisme reconstruit les albumines de son espèce.

4° Quand, à la suite d'une digestion incomplète, une albumine pénètre dans le milieu intérieur de l'organisme, c'est ce milieu intérieur qui doit parachever la digestion, et les albumines ou colloïdes introduits dans le milieu intérieur par voie hypodermique, intraveineuse, intracellulaire, se trouvent excatement dans le même cas.

5° Les cellules, tissus, organes et appareils du milieu intérieur de l'organisme ne sont adaptés que dans une certaine mesure à cette fonction de digestion et ne peuvent transformer en un temps donné qu'une quantité déterminée d'albumines ou de colloïdes étrangers. Aussi chaque fois qu'une cellule auro fixé une quantité de la substance à digérer supérieure à celle qu'elle peut digérer facilement (phénomène de surcharge), il y aura indigestion intracellulaire, d'où trouble dans les fonctions vitales et état pathologique de la cellule, surtout parce qu'elle ne possède pas de système d'évacuation commode pour le surplus non digestible. C'est le cas des toxines, de la ricine, de l'abrine, des venins et de certains sérums qui sont directement toxiques pour quelques tissus.

6° La digestion intracellulaire ne peut s'expliquer que par une attraction et une fixation de la substance à digérer par une substance de la cellule, c'est à dire par une affinité chimique entre ces deux substances qui a pour résultat la formation d'un composé nouveau ; quand cette opération n'est pas pathogène pour la cellule, celle-ci reproduit et multiplie la substance qu'elle a perdue dans cette combinaison. C'est de cette façon qu'on peut se représenter la formation de l'anticorps en excès.

7° La présence dans l'organisme de l'anticorps en excès, dans les cellules et dans le sang, qui peut par ellemême provoquer certains

troubles fonctionnels (tuberculose) augmente la capacité digestive de l'organisme pour l'antigène correspondant, mais rend en même temps la digestion plus rapide, plus tumultueuse, et aloros deux cas différents peuvent se présenter :

(*a*) Ou bien la digestion se fait en un temps et, dans ce cas l'antigène, transformé d'emblée en un produit assimilable ou éliminable, devient complétement neutre pour l'organisme. Le composé anticorps-antigène ne provoque aucun trouble : diphtérie, tétanos, colloïdes non antigènes.

(*b*) Ou bien la digestion se fait en deux phases successives : coagulation et dissolution du coagulum. Dans ce cas, la formation brusque du coagulum provoquera des manifestations pathologiques immédiates ou tardives, chaque fois que la quantité d'antigène sera supérieure à la capacité de dissolution rapide du coagulum (septicémies et albumines hétérogènes).

8° Tous les symptômes pathologiques causés par un antigène, qui se manifestent dans l'organisme au moment où celuici contient l'anticorps correspondant en excès sont donc de nature anaphylactique.

Une crise d'anaphylaxie n'est donc pas autre chose qu'une crise d'indigestion.

Les troubles sont provoqués par une brusque rupture de l'équilibre normal entre l'état de *gel* et *sol* des colloïdes qui entrent dans la composition des cellules et du sang.

La gravité des troubles provoqués par ces réactions est déterminée :

(*a*) par les quantités et les proportions des réactifs ;

(*b*) par la concentration des réactifs et, par conséquent, la durée de la réaction ;

(*c*) par la localisation intracellulaire et intravasculaire des lésions ;

(*d*) par l'effet secondaire et souvent tardif de ces lésions sur l'economie générale.

Dans les maladies infectieuses spontanées, les réactions intravasculaires sont toujours moins dangereuses que les réactions intracellulaires, parce que ces dernières peuvent avoir pour conséquence la destruction des cellules et, par conséquent, des lésions graves et durables des tissus. Les réactions intravasculaires peuvent donc être considérées comme thérapeutiques par rapport aux réactions intracellulaires.

9° La raison d'être de la production des anticorps en excès résulte de l'obligation dans laquelle se trouve l'organisme de transformer en

sels les colloïdes antigènes qui ont pénétré par une voie quelconque dans son sang et dans ses tissus.

10° Le mécanisme de cette digestion est encore inconnu, mais on peut supposer d'après l'exemple des transformations des arsenobenzènes que, dans certains cas (toxines), l'anticorps est une substance qui bloque les liaisons qui réunit les amines en granules colloïdaux et transforment ainsi un colloïde en sel neutre pour l'organisme; que, dans d'autres cas, il y a d'abord fixation par les molécules du granule colloïdal d'une substance qui réunit les granules entre eux pour former un coagulum ou un précipité et, ensuite, d'une autre substance qui dissout le précipité et bloque les liaisons des molécules.

Il est à noter, en passant, que la même substance peut coaguler ou dissoudre suivant les proportions des réactifs.

Ainsi, en deux mots :

L'état pathologique dans les maladies infectieuses est dû à l'anaphylaxie.

L'anaphylaxie est une indigestion intracellulaire ou intravasculaire, ou les deux à la fois.

L'indigestion est l'impossibilité pour l'organisme de transformer rapidement les colloïdes-antigènes en cristalloïdes.

Elle peut être intravasculaire et alors les troubles sont immédiats et rapides : choc anaphylactique ;

ou bien intracellulaire, et alors les troubles et les lésions qui en résultent peuvent être plus ou moins tardifs et durer des heures, des jours ou des dizaines d'années (tuberculose, lèpre, syphilis) : anaphylaxie chronique.

DEMONSTRATION OF MICROCOCCI IN THE BONES IN RICKETS AND SCURVY

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Additions to our knowledge of the factors concerned in the production of scurvy are being made as the subject is studied from various standpoints. That an unsuitable diet lays the foundation for the development of scurvy is generally accepted. Just what constitutes this unsuitability is one of the questions for which an answer has long been sought. As to the condition produced in guinea-pigs on certain diets, McCollum says that "only those diets which produce feces of a character readily eliminated will relieve or prevent the disease." He was able to prevent the development of scurvy in guinea-pigs fed on oats and milk by giving with it a liberal amount of liquid petroleum, and also to effect a complete cure in animals in advanced stages without change of diet by the use of petroleum. McCollum therefore concludes that scurvy in the guinea-pig is the result of the retention of feces, but whether this is the case in human scurvy or not is unknown. As to the explanation of the structural changes present he is unable to make any statement.

Hess,[1] from a study of necropsy records of cases of scurvy and from clinical records of his own cases, concludes that in human scurvy constipation plays a secondary rôle. He believes that a faulty diet permits intestinal bacteria to elaborate toxic substances which may not always be alike; he remarks the striking susceptibility to infections and thinks some of the hemorrhages are secondary while others are of scorbutic origin.

The difficulty in the way of arriving at the etiology of scurvy and allied conditions may perhaps be due to their dependence on more than one factor. It seems quite possible that the unsuitable diet may bring about a condition in the body which renders it susceptible to infectious agents of low virulence, which under other conditions would not occur. To study the problem from this view-point seems warranted.

Important as the diet must be in any investigation of the etiology of scurvy, bacteriologic and microscopic studies should not be neg-

lected. A very careful study[2] of the lesions produced in experimental scurvy lead to the belief that bacteria in some way were related to the lesions. That they are the cause of the changes needs further demonstration, but that they are present is evidenced both by the results of cultures[3] and the microscopic examination of the tissues.

I have had the opportunity to study the changes in bones, presenting signs of rickets or scurvy, from different sources. The material includes bones from 3 human cases, tissues from 5 puppies with mild evidences of scurvy, and from 1 guinea-pig with rather advanced lesions, apparently developed spontaneously under ordinary conditions. A comparison of the changes found in the different cases may be of interest.

The human specimens were obtained through the kindness of Dr. E. R. LeCount and Dr. A. M. Moody. In two of the cases there were well defined rachitic changes in the bones.

CASE 1.—A colored child, 13 months old, was admitted to St. Luke's Hospital, Feb. 2, 1916, to the service of Dr. Brenneman. A diagnosis of gastro-enteritis and rickets was made. The child died Feb. 11.

Extracts from the Postmortem Record: The anterior fontanelle is 3 cm. long by 2.7 cm. wide. The two lower central incisors are partially erupted. There are no other teeth. There is a marked rachitic rosary and marked enlargement and deformity of the heads of the long bones. The abdomen is distended. The mesenteric lymph nodes are distinctly enlarged. The epiphyseal lines of the long bones as well as the ribs are irregular and very much broadened. They contain an increased amount of cartilage. On microscopic examination the margins of the costal cartilage are very irregular and the zone of ossification broad. The diameter of the rib in this region is about twice the normal. A rather large amount of osteoid tissue is present particularly at the periphery where it apparently replaces portions of the compact bone. Near the costochondral junction and for a considerable distance from it, the marrow cells are much diminished in numbers, small hemorrhages are numerous, as are also small amounts of fibrin. In sections stained by Gram's method and also by methylene blue, cocci are found in small areas of degeneration, located here and there along the margin of the bone and cartilage and seemingly always involving a blood vessel. The organisms are found both within the vessels and in the surrounding tissues. Farther from the cartilage the marrow is more normal in appearance. It is very hyperemic and many eosinophil cells are scattered through it.

CASE 2.—Child, 1 year old, admitted to Cook County Hospital, April 24, 1916, to the care of Dr. Michael. According to the clinical record the child was breast-fed the first 3 months; after that Borden's milk was given. The child had always been fretful and the last 2 weeks had coughed a good deal. There were râles posteriorly on both sides, temperature 105 F., respiration rapid, and patches of bronchial breathing. The child died 5 days after entrance.

Anatomic Diagnosis: Rachitic osteochondritis; "pigeon breast," hyperplasia of the spleen, cervical, tracheobronchial, periaortic and mesenteric lymph glands;

[2] Jackson, L., and Moore, J. J.: Jour. Infect. Dis., 1916, 19, p. 478.
[3] Jackson, L., and Moody, A. M.: Ibid., p. 511.

marked general anemia; slight cloudy swelling of the kidneys; marked fatty changes in the liver; atelectasis and compensatory emphysema of the lungs; localized, left, fibrous pleuritis.

Sections of the ribs from this case prepared for microscopic examination include the costochondral junctions which are greatly increased in diameter. Large, irregularly rounded masses of cartilage extend for some distance into the marrow cavity, and for even greater distances at the periphery of the bone. The compact bone in these places has a fragmented appearance and is partially replaced by osteoid tissue. The bone marrow for a distance of 1.5 cm. from the cartilage is almost completely replaced by fibrous tissue and even where more normal marrow is found it occurs in larger or smaller patches surrounded by fibrous tissue. Scattered through the bone marrow spaces are many small hemorrhages. In some of these and also in the surrounding tissue and in occasional thrombosed vessels, cocci are found and sometimes they were quite numerous. In sections stained with phosphotungstic acid hematoxylin, small amounts of fibrin are found in these locations.

Case 3.—The material was obtained from a colored infant, dying 3 hours after birth. The Wassermann reaction of the mother was negative and no spirochetes were found in Levaditi preparations of the tissues of the infant. The only gross changes were enlargement of the costochondral junctions and the ends of the long bones. The ribs were increased in width at the costochondral junctions and the epiphyseal junctions were somewhat widened. In sections of the rib the cartilaginous margin is very irregular. Prolongations of cartilage extend down into the marrow cavity and at the periphery these form large rounded masses. There is a marked decrease in the normal marrow cells in the region bordering on the cartilage and for about a centimeter from it, and a rather marked increase in the amount of fat. There are a number of small hemorrhages present. In the upper portion of the tibia the changes are not so marked. The epiphyseal line is fairly distinct but somewhat irregular. There are, however, hemorrhages into the bone marrow and in places a rather marked degenerative process in the cells and some of the bony trabeculae. Cocci were occasionally found along the margins of bone and within and about blood vessels.

The prominent features in these cases are: increased width at the costochondral and epiphyseal junctions; a broad zone of ossification; replacement of bone marrow cells by fibrous tissue and of bone by osteoid tissue; many hemorrhages, and small typical regions of degeneration in which small cocci often were found.

The 5 puppies examined were from two litters, 1 from the first and 4 from the second. The puppies of the first litter, when purchased, were thin but otherwise appeared normal. Although well fed and cared for, they gradually became thinner and finally died. At postmortem examination of the last one of these animals, well marked enlargements of the costochondral junctions of the ribs were discovered. Doubtless intestinal worms were present in this case but were not found on account of failure to examine the intestines. This dog at the time of death was about 4 months old and had received no treatment. The members of the second litter appeared healthy and well nourished when purchased, but they also gradually lost flesh; two of them died and two were killed. In all of these the intestinal tract and stomach contained numerous worms (Ascaris camis), and in all of the puppies there were mild changes in the bones. Other-

wise there were no noteworthy gross changes. The age of these animals was about 2 months and they had received no treatment.

The bone changes in all of these animals were mild and necessarily of short duration. The alterations found in the one mentioned first were slightly more advanced but typical of all and will therefore be described in some detail. There is rather abrupt swelling at the costochondral junction to about twice the normal diameter. The margin of the cartilage is slightly irregular and there is a definite line of demarcation between the cartilage and bone. There is slight rarefaction of the marrow in this region, more marked at the periphery, together with small hemorrhages and edema. There are numerous small lesions in the bone marrow in which are degenerative changes in the endothelial and marrow cells, fibrin formation and often hemorrhage. These lesions are in close relationship to blood vessels and bone especially along the edge of the compacta and between the periosteum and the bone. In this type of lesion cocci are quite frequently found. It should be mentioned here that the regional lymph glands in these dogs are hemorrhagic and microscopically present an appearance quite similar to those found in experimental scurvy in guinea-pigs.

It will be noted that the changes in the puppies are apparently milder and of shorter duration than in the human cases, but that in other respects there is a rather close resemblance.

The guinea-pig examined was one that seemingly had contracted the disease spontaneously. Three weeks before it was killed the pig had been injected with urine from a case of nephritis. No swelling of the joints was noticed at the time of injection, but judging from the appearance of the lesions the beginning of the process in the joint must have preceded the injection by a considerable length of time. At necropsy it was noticed that one of the wrist joints was decidedly enlarged and that the costochondral junctions were swollen. There were hemorrhages about the joint and the corresponding axillary lymph glands were enlarged and hemorrhagic. So far as known this pig had always received the ordinary diet for guinea-pigs. Examination of microscopic preparations of the wrist joint revealed the presence of extensive alterations in the ends of both the ulna and radius. The marrow was almost entirely replaced by a well developed fibrous tissue and at the periphery of the bones in the region of the epiphysis there were still large areas of necrosis. There was a decrease in the amount of change present as the proximal ends of the bones were approached. Even in these bones where the changes appeared oldest there were the typical small lesions indicating that the process was still active. In the small bones of the wrist the process appeared more recent. There was little fibrous tissue replacing the marrow but there was much hemorrhage and edema. The changes in the rib examined were mild. It is apparent that the changes just described are identical with those found in moderately severe cases of experimental scurvy.

SUMMARY

In the cases considered in this paper there appear rather marked differences in the acuteness and severity of the process. The appearance in the human cases is that of a subacute process continued over a rather long period of time. There are no large amounts of necrosis such as are seen in the guinea-pig, although this feature may have

been more prominent at some stage in the disease. In fact, they present much the same appearance as some of the older healing cases of experimental scurvy in guinea-pigs. The changes in the bones of the puppies are the mildest and suggest a subacute process operating for a shorter length of time and in this respect resemble more closely the changes found in the infant.

The location of the alterations is the same in all the cases, the most noticeable feature being the increase in diameter at the junction of cartilage and bone with most pronounced changes at the periphery, and the relation of changes to blood vessels and bone.

The changes invariably present are hemorrhage, edema, and more or less degeneration of the endothelial and marrow cells and bone.

The similarity in the nature of the process producing the alterations in all of these cases is quite striking. Wherever it is active there is more or less degeneration of cells with fibrin formation, usually involving a blood vessel and often accompanied by hemorrhage. It is in these places that micrococci are often seen. A distinctive feature of all of these cases is the absence of any marked cellular exudate in relation to the lesions at any stage of the disease. There is first degenerative changes in the cells and bone followed by disappearance of normal cells and replacement by fibrous tissue.

While we cannot as yet state positively that the lesions described are due directly to the action of the micrococci so frequently found in connection with the lesions, and particularly the fresh ones, the presence of cocci appears at least significant. Even should we subsequently decide that these organisms are but secondary invaders it still seems that the evidence presented by microscopic preparations of the lesions argues for the presence of some infectious agent, and emphasizes the need of further work on the bacteriology of these conditions.

OBSERVATIONS ON THE BACTERIOLOGY AND IMMUNE REACTIONS OF RUBEOLA (MEASLES) AND RUBELLA (GERMAN MEASLES)

PLATE 14

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In a previous article I[1] have described a micrococcus isolated in anaerobic cultures from the blood of measles patients. The cultures were made during the preeruptive and early eruptive stages of the disease, at which time the virus of measles is present in the blood (Hektoen,[2] Anderson and Goldberger[3]).

The blood was drawn with a sterile syringe from the vein at the elbow, after carefully washing with alcohol or tincture of iodin and alcohol. Generally, whole blood was added to the medium, but sometimes it was collected in sodium citrate solution. The cultures were incubated at 36 C.

Anaerobic cultures were made in semicoagulated horse serum; on anaerobic Krumwiede[4] plates as used by Dick and Henry[5] in blood cultures in scarlet fever; in ascitic fluid and horse serum alone or with plain and dextrose broth, with and without tissue. Anaerobic cultures were made of laked blood and lately of whole blood in ascitic dextrose agar shake cultures as used by Plotz[6] in isolating the typhus bacillus.

The coagulated horse serum was prepared by heating the serum, about 10 c c in each tube, at 56 C. for one hour on three successive days, the temperature at last being raised slowly to 70 C. and kept there until the serum became semicoagulated. The tubes were incubated four or five days before being used. About 1 c c of blood was added to each tube directly from the syringe and forced toward the bottom of the tube with a sterile pipet. When the cultures were examined, some of the contents from different parts of the tube were removed with a pipet, and smears and subcultures made.

[1] Jour. Am. Med. Assn., 1917, 68, p. 1028.
[2] Jour. Infect. Dis., 1905, 2, p. 238.
[3] Pub. Health Rep., 1911, 26, 847, 887. Jour. Am. Med. Assn., 1911, 57, p. 113.
[4] Jour. Infect. Dis., 1913, 12, p. 199.
[5] Ibid., 1914, 15, p. 85.
[6] Ibid., 1915, 17, p. 1. Baehr: ibid., 1917, 20, p. 201.

In using the Plotz method only 0.5 c c of blood was added to the ascitic dextrose agar on account of the difficulty of otherwise seeing the colonies. Large test tubes containing 20 c c of dextrose agar were employed so that the contents could be mixed without being poured into another tube. Aerobic cultures were made in the fluid mediums mentioned. Subcultures from the various inoculations were made on ascitic goat blood-agar slants, in semicoagulated horse serum and various fluid mediums. The original cultures and subcultures were made anaerobic by the use of pyrogallic acid and sodium hydroxid, the stoppers being paraffined. Occasionally sterile fluid paraffin was used on fluid cultures.

Successful cultures were not obtained in any of the anaerobic fluid mediums or in the aerobic cultures. The largest number of positive cultures were obtained in semicoagulated horse serum and the whole blood ascitic dextrose-agar shake cultures. The anaerobic plates and the hemolyzed blood ascitic dextrose shake cultures were fairly satisfactory.

Blood cultures were made from 50 patients in the preeruptive and eruptive stages and from 5 when the rash was fading. In every instance it concerned true measles — rubeola. A small micrococcus was observed in the original anaerobic cultures in 42 of the early cases. The cocci were found in all of the last 15 cases studied. In the earliest culture, obtained a few hours after the appearance of the Koplik spots and 24 hours before the eruption, there were only a few organisms. Cocci were present in large numbers in the cultures of three patients made a few hours before the appearance of the exanthem; two in ascites dextrose shake cultures showed innumerable colonies. Similar shake cultures made in a case when the rash was just appearing showed about one-half as many colonies. The organisms now become less numerous and disappear from the blood as soon as the rash begins to fade.

Twenty-eight of the 42 positive cultures grew slightly for two or three generations only. One strain remained strictly anaerobic, growing only on blood agar and not in fluid mediums. Thirteen strains became aerobic in the second generation and grew fairly abundantly.

Growth appears in the original tubes in from 1-22 days, generally in from 5-15 days. The cultures in which the organisms were first found in the second and third week may have grown out earlier and not been observed. Subcultures generally grow in 24 hours after growth is established. Growth in the strictly anaerobic strain occurs on about the ninth day.

Successful subcultures on plain blood agar plates show small greenish colonies with regular margins giving generally a decidedly green color to the medium.

The growth on dextrose blood agar is more profuse and more moist and gives the culture a burnt appearance. Some strains produce a slight hemolysis after cultivation. Four of the strains gave larger, moister colonies. These four do not decolorize milk; some of the others produce acidity and some coagulation. They all grow on plain agar as a colorless growth. In ascitic dextrose broth the growth is flocculent, the fluid often remaining clear. They grow a little in plain broth. All ferment dextrose and saccharose and all but one maltose. An occasional strain ferments mannite and lactose. None ferment inulin, salicin and raffinose. None are soluble in bile. None liquefy gelatin.

In smears from the original culture, the organism appears as a very small, round, sometimes flattened diplococcus or in short chains, sometimes as clumps of cocci of varying sizes. In the coagulated horse serum they are so small and sometimes so few in number that considerable search is necessary to find them. They are perfectly distinct, however, when they are found. In subcultures the organisms appear larger, generally as a diplococcus, sometimes in short chains, but sometimes in long chains or in clumps like staphylococci. The cocci at the ends of chains are occasionally smaller than those in the center. This is especially noticed in the anaerobic strains. Large forms are occasionally seen. The organism is gram-positive and stains with ordinary stains. Carbol-gentian-violet was used in staining the original cultures on account of its penetrating power.

A similar diplococcus was isolated on aerobic blood plates from the throats of all the patients examined and was the organism most frequently found in anaerobic cultures from the nose and eye. It was isolated in pure culture from discharge from the ear. The organism was not found in similar blood cultures from five measles patients with fading eruptions, five normal persons, two suspected measles cases and three scarlet fever patients. It is not described by Dick and Henry in their exhaustive work on anaerobic blood cultures in scarlet fever.

Cultures of the diplococcus pass through Berkefeld N filter. They resist drying 24 hours, none being alive in 48. Swabs were soaked in a horse serum dextrose broth culture, allowed to dry in sterile test tubes at room temperature for varying periods and then cultured. The organism is killed by heating at 57 C. for 45 minutes. It resists a temperature of —2 C. for at least 14 days, and 50% glycerol 2 months at least.

In scarlet fever, Dick and Henry found that at the height of the attack a variety of bacteria are present in the blood. The same occurs in measles. Before the eruption the diplococci I describe were found

alone in the blood and alone also in twenty-one cases soon after the appearance of the exanthem. At the height of the fever, however, various other bacteria were isolated from the blood in twenty cases: Aerobic (ten cases) and anaerobic diphtheroid bacilli (twelve), filamentous organisms (six), gram-negative spirilla (four), a black pigment producing bacillus (one), spore forming large bacilli (four), and staphylococci (three). With the exception of the gram-negative spirilla, similar organisms were also cultivated from the throat, nose or eye. It is possible that some of these growths came from contaminations, but surely not all.

The special points of interest in this work were: the almost constant presence of a diplococcus in the blood of measles patients during the pre-eruptive and eruptive stages; its presence alone and in large numbers before the apearance of the eruption and alone also in a large proportion of cases at the height of the infection, and its presence at the same time in the throat, nose and eyes.

This coccus is pathogenic for the rabbit, young dog, mouse, rat, and monkey. The sediment of about 20 c c of horse serum dextrose broth cultures killed a rabbit in 24 hours, the organism being isolated from the heart blood and kidney. No lesions were found. The growth from one blood-agar slant killed a rabbit in 24 hours producing hemorrhages in the heart valve and pneumonia of one lung. Proportional doses killed a mouse in 24 hours and made a young dog and rat ill, but did not kill. A monkey (giant rhesus) was inoculated intravenously with the sediment of 100 c c horse serum dextrose broth. It was very ill the next day and slightly ill for several days, but at no time did it show any symptoms of measles. The leukocyte count rose from 14,000 to 24,000 the day following the inoculation, falling to 7,000 on the 11th day—the same blood picture as produced by the inoculation of measles blood (Hektoen and Eggers,[7] Lucas and Prizer,[8] Tunnicliff[9]). This reaction is considered due to the coccus, because two other monkeys· inoculated with equaly large numbers of spirilla isolated from the blood of measles patients produced no such change.

Cocci similar to those found in these blood cultures were found in sections of the lung and kidney in one fatal case of measles; in the lung and bronchial lymph gland of another, and in the gangrenous tongue of a third. They appear singly, in pairs and chains. Some are found inside of leukocytes. The cocci in these sections vary considerably in size (Fig. 3).

Similar blood cultures were made from ten rubella patients. The anaerobic Krumwiede plates, as used by Dick and Henry, gave the

[7] Jour. Am. Med. Assn., 1911, 57, p. 1833.

[8] Jour. Med. Research, 1912, 26, p. 181.

[9] Jour. Infect. Dis., 1912, 11, p. 474.

most satisfactory results. The inverted bottom of a petri dish is placed inside the inverted top and sterilized in a towel, by dry heat. About 1 c c of freshly drawn blood is added to 9 c c of melted agar, and after thoroughly mixing, this is poured into the inverted top of the petri dish and the inverted bottom laid directly on the melted agar. Boiling paraffin is used to seal the space between the plates. Plain or dextrose agar without ascitic fluid was used. A fairly large diplococcus was observed in the anaerobic cultures in seven of these ten cases. Two cultures made just as the eruption was appearing did not grow in subculture, those from five others, made at the height of the infection, grew fairly abundantly. Two negative cultures were taken from patients at the onset of the attack and one as the eruption was fading. Only one or two colonies were present on each plate in the successful cultures. Similar diplococci were also found in ascitic-dextrose shake and in semicoagulated horse serum cultures. Single colonies of the same diplococci were found in two cases on the aerobic plates. A diphtheroid bacillus was isolated twice from anaerobic cultures. Throat smears and cultures on aerobic blood-agar plates from six cases showed diplococci similar to those isolated from the blood.

The colonies appear on the anaerobic plates after 1-5 days' incubation, as indistinct greenish-brown colonies. The organism becomes aerobic in the second generation. On plain blood-agar aerobic plates the colonies are small, dull and green with regular margins. They become larger and moister on cultivation. Some strains show slight hemolysis. They produce a burnt appearance on dextrose blood agar. All ferment maltose, dextrose, lactose, salicin, and saccharose. Two strains also ferment inulin, mannite and raffinose. All but one liquefy gelatin, and produce a delicate colorless growth on plain agar. All grow a little in plain broth, one produces a sediment. They grow well in dextrose broth. The organisms are not soluble in bile.

In smears these organisms appear fairly large (1-2 microns in length), in pairs or chains with pointed ends generally, the body often being elongated, occasionally they occur in clumps. The pointed ends disappear on cultivation. They possess a capsule. The diplococcus is gram-positive and stains with the ordinary stains. The cultures of this organism do not pass through a Berkefeld N filter. They resist drying five days. They are killed by heating at 65 C. for one-half hour. They resist a temperature of —2 C. and 50% glycerin for 6 months at least. They are not pathogenic for rabbits.

Various immunity tests have been made with the cocci isolated from rubeola and rubella to determine if any antibody reaction could be demonstrated. So far opsonins have been the only antibodies found in any appreciable amount. Both the Wright and the dilution method have been used with similar results. In the dilution method the normal and immune serums are diluted with physiologic sodium chlorid solution to determine the point of opsonic extinction — the point at which induced phagocytosis recognizably exceeds phagocytosis with salt solution. Normal human leukocytes were used. The specimens were incubated at 37 C. for 15 minutes and the smears then stained with carbol-thionin. Typical strains of Streptococcus viridans from the throat, pneumococci, and the coccus from poliomyelitis were also studied to determine if there was any specificity in the opsonic action.

The serum of twelve measles patients was examined, in six only once as the eruption was beginning to fade, in six usually daily throughout the course of the disease. Four of these serums showed a negative phase of 1, 2, 2, and 5 days' duration, respectively. A distinct rise in opsonins for the measles organism occurred in each case as the symptoms subsided and the eruption disappeared. This rise lasted from 1-4 days. The point of opsonic extinction for normal blood was 1 : 30; for measles serum 1 : 15 during the negative phase and from 1 : 60 to 1 : 340 during the rise. The various strains isolated from the blood, throat, nose, eye and ear of measles patients gave similar results.

Two of these patients were examined daily for opsonins with the measles coccus and also with the coccus from the blood of a rubella patient. While the opsonins changed for the measles coccus, they remained within the normal limits for the rubella organism. Five rubella patients showed just the reverse opsonic content in the blood, showing typical opsonic changes for the rubella coccus and none for the measles organism. With the rubella coccus, the point of opsonic extinction for normal serum was 1 : 30; for rubella serum 1 : 15 during the negative phase and 1 : 120 to 1 : 240 during the rise. The negative phase and rise both lasted from 1-3 days. The point of opsonic extinction with the measles coccus with both normal and rubella serum was about 1 : 30. There were no changes in the opsonic content of the blood of either measles or rubella patients for the Streptococcus viridans, pneumococcus or the poliomyelitis coccus, in any of the serums examined. The results indicate that specific opsonins for the rubeola

and rubella cocci develop during the course of these diseases, disappearing with the recovery of the patient. After cultivation for a variable length of time both the rubeola and rubella cocci lose their original morphological characteristics, but retain their specific immunological properties.

SUMMARY

Diplococci may be isolated from the blood of patients with rubeola and rubella. While the two organisms are similar in their cultural characteristics, they differ in size, shape, capsule formation, filtrability, viability, virulency, and immunity reactions.

EXPLANATION OF PLATE 14

Fig. 1.—Forty-eight hour growth on ascitic goat blood agar of diplococcus isolated from blood of rubeola patient; Gram stain; × 1000.

Fig. 2.—Twenty-four hour growth on goat blood agar of diplococcus isolated from throat of rubeola patient; Gram stain; × 1000.

Fig. 3.—Section through lung of rubeola patient showing cocci in chains; Gram stain; × 1000.

Fig. 4.—Twenty-four hour growth on goat blood agar of diplococcus isolated from blood of rubella patient; Gram stain; × 1000.

Fig. 5.—Twenty-four hour growth on goat blood agar of diplococcus isolated from throat of rubella patient; Gram stain; × 1000.

PLATE 14

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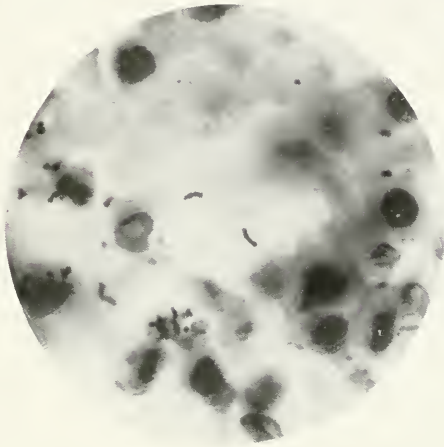
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THE TOXICITY OF PNEUMONIC LUNGS *

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In lobar pneumonia the toxemia is ascribed largely to toxic sub-
stances from virulent pneumococci. Numerous investigations have
shown that pneumococci do not produce appreciable amounts of
soluble toxin in cultures, although the work of Neufeld and Dold,[1]
Rosenow[2] and Cole[3] indicate that a toxic substance is produced from
the cocci or contained within them which, when liberated, is capable
of producing death in animals with symptoms and lesions suggestive
of anaphylaxis. Cole regards this pneumotoxin as preformed in the
bacterial cell; the studies of Cohen, Weiss and Kolmer[4] generally
confirm these observations, although they experienced considerable
difficulty in the preparation of the pneumotoxin. While this toxic
substance may be elaborated during lobar pneumonia in sufficient
amounts to account in large part for the toxic symptoms in addition
to probably influencing enzymic processes, our experiments show that
its production in vitro is quite irregular, that large numbers of virulent
pneumococci are required for its production, and that relatively large
amounts of it are required to produce intoxication of animals. When
it is remembered that sections of pneumonic lungs frequently show
few pneumococci, it is questionable whether there is a sufficient amount
of pneumotoxin produced in lobar pneumonia to account in whole for
the toxemia of this disease.

With these considerations in mind we have studied the exudate
in lobar pneumonia as a further source of toxic substances. While
the investigations of Schenck,[5] Dold,[6] Roger,[7] Wright,[8] and Riesman

[1] Berl. klin. Wchnschr., 1911, 48, p. 55.
[2] Jour. Infect. Dis., 1911, 9, p. 190.
[3] Jour. Exper. Med., 1912, 16, p. 644.
[4] Jour. Infect. Dis., 1918, 22, p. 476.
[5] Ztschr. f. Immunitätsf., O., 1914, 22, p. 229.
[6] Ibid., p. 561.
[7] Arch. d. Med. Exp. et d'Anat. Path., 1911, 23, p. 37.
[8] Proc. Roy. Irish Acad., 1891-1893, 2, p. 117.

and Kolmer[9] have shown that extracts of various tissues, notably lung tissue, are highly toxic on intravenous injection and produce intravascular coagulation, increase in the coagulation time, fall of blood pressure, etc., yet the pneumonic exudate as a source of toxic substances has not received the attention which we believe it deserves. Riesman[10] has recently drawn particular attention to this subject in a discussion on what he calls the "cellular factor in infectious diseases," and states that in pneumonia the bacterial toxemia has been overemphasized to the exclusion of everything else, whereas it is not improbable that some of the general symptoms are due to the exudate, independently of the bacteria. The work of Rosenow and Arkin[11] indicates that the exudate in lobar pneumonia is toxic in that extracts of pneumonic lungs in salt solution injected intravenously in dogs produced blood pressure and respiratory disturbances strikingly similar to those of protein anaphylaxis in general.

The Toxicity of Extracts of Normal and Pneumonic Lungs

Lungs secured soon after death, and in the stage of gray hepatization, were cut in small pieces, washed and the consolidated parts passed through a meat grinder; this material was then subjected to high pressure in a Buchner hydraulic press, the juice centrifuged at high speed, and the fluid filtered through sterile paper. Almost invariably these extracts contained pneumococci and other bacteria capable of killing animals in small doses; for this reason they were sterilized with phenol up to 0.5% or the fluids were recentrifuged for an hour at high speed and heated at 56 C. for 30 minutes. Marked differences in toxicity followed these two methods of preparation, the first method yielding products which were invariably more toxic than the second. The more ideal method of securing the toxic substances of the lung tissue and exudate by passing the juice through a sterile Berkefeld filter to remove the pneumococci, was unsuitable inasmuch as the toxic substances were largely removed by the filtration.

The fatal toxic dose of pneumonic lung extract prepared by phenol sterilization was about 4 c c per kilo of weight when injected intraperitoneally in mice; the toxicity of fresh extract sterilized by thorough centrifuging and heating at 56 C. for 30 minutes, was between 15 and 25 c c per kilo of weight (Table 1).

By intravenous injection in guinea-pigs, doses of phenolized extract greater than 0.5 c c per kilo of weight were almost invariably fatal within a few minutes producing convulsions and dyspnea similar to acute anaphylactic intoxication (Table 2). Table 2 also shows that intravenous injections of the phenolized extracts in rabbits usually resulted in death within a few minutes in doses greater than 0.12 c c per kilo of weight. Repeated intramuscular injections of these extracts in rabbits, produced fever and rapid loss of weight; doses greater than 0.8 c c per kilo of weight usually produced death after 2 or 3 injections.

9 Trans. Assoc. of Amer. Phys., 1912.
10 Trans. College of Phys. of Phila., 1914, 36, p. 271.
11 Jour. Infect. Dis., 1912, 11, p. 480.

TABLE 1

Toxicity of Extract of Human Pneumonic Lung for Mice by Intraperitoneal Injection

Weight in Gm.	Dose per Kilo in c c	Kind of Extract	Results
17	25	Heated	Died in 48 hours—heart blood sterile
24	15	Heated	Survived
25	7.5	Heated	Survived
21	5	Heated	Survived
19	4	Heated	Died in 48 hours—heart blood sterile
19	2	Heated	Survived
13	1	Heated	Survived
17	0.5	Heated	Died in 24 hours—heart blood sterile
13.5	16	Phenolized	Died in 24 hours
20	8	Phenolized	Died in 24 hours
15	4	Phenolized	Died in 24 hours
20.5	2	Phenolized	Survived
20	1	Phenolized	Survived
20.5	0.5	Phenolized	Survived

TABLE 2

Toxicity of Extract of Human Pneumonic Lung for Guinea-Pigs and Rabbits by Intravenous Injections

Weight in Gm.	Dose in c c per Kilo	Results
Guinea-pig 215	2.3	Died in one minute in convulsions
Guinea-pig 190	1.0	Died in convulsions in 10 minutes
Guinea-pig 225	0.7	Died in 24 hours—no immediate symptoms
Guinea-pig 195	0.5	Survived—no symptoms
Rabbit 2350	0.5	Died in one minute—convulsions
Rabbit 2200	0.25	Died in two minutes—convulsions
Rabbit 2800	0.12	Restless, survived; lost weight

That the toxicity of the extracts cannot be ascribed entirely to the cellular and serofibrinous exudate, is shown by the toxicity of extracts of normal human lungs prepared in the same manner and of equal weights of the respective tissues. The anterior lobes of normal human lungs secured shortly after death, extracted and phenolized in the same manner as the pneumonic tissues, proved about one-half to one-fifth as toxic for various animals after injection by various routes as similar extracts of pneumonic tissue (Table 3).

TABLE 3

Comparative Toxicity of Extracts of Normal and Pneumonic Human Lungs

Extract of Normal Lung			Extract of Pneumonic Lung		
Weight in Gm.	Dose in C C per Kilo	Result	Weight in Gm.	Dose in C C per Kilo	Result
21	20	Died in 48 hours	20	10	Died in 24 hours
22	15	Died in 24 hours	24	8	Died in 24 hours
16	10	Survived	23	4	Survived
10	8	Survived	17	1	Survived

As the extracts of pneumonic lungs were more toxic than similar preparations of normal lungs, we undertook to analyze this phenomenon and to determine, if possible, to what element, pneumotoxin—that is, the poison derived from autolyzed pneumococci—or poison of the inflammatory exudate. this toxicity is due.

Dogs were infected with concentrated broth cultures of virulent pneumococci by intrabronchial insufflation (Lamar and Meltzer).[12] By means of a proper catheter, 20 c.c. of a 12-24-hour broth culture of virulent Type I pneumococci were injected intrabronchially; 0.000,001 of 24-hour culture killed mice. In each experiment a second dog was given an equal amount of a thick sterile emulsion of aleuronat or a sterile saturated aqueous solution of commercial peptone, in exactly the same way. Forty-eight hours later these dogs as well as a normal control, were killed, the lungs extracted and prepared by the two methods described. With virulent pneumococci, extensive areas of consolidation, as described by Lamar and Meltzer, were invariably observed; aleuronat and peptone produced more irregular consolidation and the lesions resembled more closely those described by Riesman and Kolmer as due to pneumococci of lesser virulence, and by Wollstein and Meltzer as produced by heat-killed pneumococci and sterile broth. The results, while irregular, indicated clearly, as shown by Table 4, that the sterile extracts of consolidated lung due to virulent pneumococci were more toxic than extracts of aleuronat consolidation and of normal lung; likewise extracts of aleuronat exudate were more toxic than the extracts of normal lung (Table 5).

TABLE 4

Comparative Toxicity of Phenolized Extracts of Normal and Consolidated Dog Lungs for Mice on Intraperitoneal Injection

Extract of Pneumococcus Consolidated Lung			Extract of Aleuronat Consolidated Lung			Extract of Normal Lung		
Weight in Gm.	Dose per Kilo in C C	Result	Weight in Gm.	Dose per Kilo in C C	Result	Weight in Gm.	Dose per Kilo in C C	Result
20	32	Died in 24 hours	19	25	Survived	20	50	Died in 72 hours
27	16	Died in 24 hours	..	..	....	15	30	Survived
20	8	Survived	..	..	....	..	..	....

TABLE 5

Comparative Toxicity of Phenolized Extracts of Aleuronat Exudate in Dog Lung and Normal Dog Lung by Intravenous Injection in Guinea-Pigs

Extract of Aleuronat Lung			Extract of Normal Lung		
Weight in Gm.	Dose per Kilo in C C	Result	Weight in Gm.	Dose per Kilo in C C	Result
215	2	Died in 24 hours	250	5	Died in 48 hours
255	1	Survived	260	4	Survived
...	..	......	250	1	Survived

The toxicity of extracts of pneumonic lung in gray hepatization and of normal lung is slightly decreased by heating the extracts at 60 C. for 1 hour; the pneumotoxin, that is, the toxic substance obtained by autolysis of virulent pneumococci, is, however, extremely thermolabile. The toxicity of one extract

[12] Jour. Exper. Med., 1912, 15, p. 133.

reheated at 60 C. for 1 hour was appreciably decreased; similar experiments with other extracts prepared by phenol sterilization showed that exposure at 56 C. for 1 hour had less effect, so that the toxic factors may be regarded as largely thermostabile.

Thorough drying of the extracts of normal and pneumonic lungs (in gray hepatization) at 19 C. by means of an electric fan, followed by emulsification of the powder in sterile salt solution and injection intraperitoneally in mice, gave a similar decrease in toxicity in experiments so conducted that the doses of dried substances corresponded to the extracts in the fluid state before drying.

Filtration of the extracts through sterile filter paper of medium density does not alter appreciably their toxicity; filtration through small unglazed porcelain (Kitasato) and Berkefeld N filters appreciably reduces the toxicity, but does not remove it entirely, as occurs on the filtration of the pneumotoxin alone through similar filters.

As shown by Cole[3] the pneumotoxin obtained by autolysis of virulent pneumococci in solutions of sodium choleate, is frequently hemolytic for the erythrocytes of the guinea-pig and other animals; in the experiments of Cohen, Weiss and Kolmer[4] it was found difficult to prepare this hemolytic substance but a sufficient number of experiments were successful to confirm Cole's observations and indicate the hemolytic nature of the endocellular toxin of the pneumococcus. Similar results were observed with extracts of pneumonic lung in gray hepatization, that is, some extracts were markedly hemolytic for guinea-pig cells and others were not hemolytic in large amounts; like the pneumotoxin, the hemolytic effect in extracts of pneumonic lung was reduced by heating and drying and usually completely removed by filtration through Berkefeld filters (Table 6). Similar extracts of normal human lung tissue were found to have little or no hemolytic effect and extracts of the exudates of lungs of dogs removed 48 hours after the intrabronchial injection of virulent pneumococci and of sterile aleuronat, were generally nonhemolytic, indicating that in fresh pneumococcus exudates appreciable amounts of hemolytic substance are not present but present in human pneumonic lungs in gray hepatization and with early autolysis of the exudate.

TABLE 6

The Hemolytic Activity of Phenolized Extracts

Extract	Dilution of Extract	Results
Extract of human pneumonic exudate	1:100	Marked lysis with 0.2 c c, complete lysis with 0.3 c c of substance
Extract of normal human lung	1:50	Very slight lysis with as much as 2.0 c c of substance
Extract of pneumonic exudate heated at 56 C. for 60 minutes	1:100	No lysis with 0.7, marked with 0.8, and complete with 0.9, c c of substance
Extract of pneumonic exudate, dried, emulsified in salt solution	1:100	No lysis below 0.5, complete lysis with 0.7 c c of substance ·
Dried extract of normal lung	1:50	No lysis
Berkefeld filtrate of pneumonic exudate	1:100	No lysis
Extract of pneumococcus exudate in lung of dog (48 hours)	1:10	No lysis
Extract of aleuronat exudate in lung of dog (48 hours)	1:10	No lysis
Extract of normal dog lung	1:10	No lysis

The nature of the hemolytic substance sometimes present in extracts of pneumonic lungs in the later stages of pneumonia, is unknown; our experiments indicate that this agent may be neutralized not only by antipneumococcus serum corresponding to the type of the pneumococcus infection, but also by normal serum, e. g., rabbit. It is probable that the lipoidal constituents of these serums act as antihemolytic agents and additional experiments indicate that the presence of lipoids in the lung extracts decrease their hemolytic activity inasmuch as the removal of lipoids from dried pneumonic tissue exudates, is followed by an increase of hemolytic activity.

Cole[13] has shown recently that the fluid of pneumococcus empyema may contain large amounts of soluble substances which have the property of neutralizing pneumococcus antibodies; since then we have had the opportunity of studying the influence of one extract from pneumonic lung in gray hepatization, obtained by grinding the tissues and by high pressure in a hydraulic press followed by thorough centrifugation and filtration through sterile paper. This extract was not heated and was used at once without preservative. A similar extract was prepared of an equal weight of normal human lung tissue. Both extracts were tested for inhibition of agglutination of Type I pneumococci by homologous antiserum (Rockefeller Institute) by the technic described by Cole. While 0.4 c c of serum diluted 1:60 produced well marked agglutination of 0.1 c c of a broth culture of pneumococci, the addition of 0.4 c c of pneumonic lung extract reduced the agglutinating influence of the serum to a dilution of 1:20 and the extract of normal lung to a dilution of 1:40 (Table 7).

TABLE 7

The Inhibition of Agglutination of Pneumococci by Antipneumococcus Serum by the Extracts of Pneumonic and Normal Lung *

Extract	Dilution of Immune Serum							
	1:2	1:4	1:8	1:20	1:40	1:60	1:80	1:120
Pneumonic lung...............	+	+	+	+	—	—	—	—
Normal lung....................	+	+	+	+	+	—	—	—
Control--salt solution..........	+	+	+	+	+	+	+	+

* Mixtures of 0.4 c c of lung extracts or salt solution with 0.4 c c of immune serum, undiluted and diluted, were made in small tubes and placed at 37 C. for 30 minutes when 0.1 c c of a 24-hour broth culture of Type I pneumococci was added to each tube; the tubes were then placed in incubation for one hour and in cold box over night.

While this result requires further work before definite conclusions can be drawn, it would appear that extracts of lung tissue and particularly of pneumonic lung in gray hepatization, contains substances capable in slight degree of neutralizing the agglutinin in antipneumococcus serum similar to those found by Cole in pneumococcus empyema fluids.

CONCLUSIONS

Salt solution extracts of pneumonic lung in the stage of gray hepatization were found to be more toxic for animals than similar extracts of normal lung tissue. The method of extraction influenced the toxicity of both extracts.

[13] Ibid., 1917, 26, p. 453.

Lethal doses of extracts of both pneumonic and normal lung injected intravenously usually produced anaphylactic symptoms.

Sterile extracts of pneumonic lung in dogs removed 48 hours after intrabronchial insufflation of virulent pneumococci, were more toxic than similar extracts of lung consolidated by intrabronchial insufflation of sterile aleuronat, and both were more toxic than extracts of equal weights of normal dog lung.

The toxicity of extracts of normal and pneumonic lung is decreased by heating, drying and filtration through Berkefeld filters.

Extracts of human pneumonic lungs in gray hepatization were frequently hemolytic for guinea-pig cells, whereas similar extracts of normal human and dog lungs and of dog lungs consolidated from intrabronchial insufflation of virulent pneumococci and sterile aleuronat, were generaly nonhemolytic. The hemolytic activity of these extracts was neutralized by antipneumococcus serum as well as by normal rabbit serum; reduced by heating and drying, and usually completely removed by porcelain filtration.

One extract of a human pneumonic lung in gray hepatization was found to partly neutralize the agglutinating activity of an antipneumococcus serum.

The nature of the toxic and hemolytic substance or substances in extracts of pneumonic lung is unknown; it is probably allied with the protein fractions and may be partly responsible for the production of the various systemic symptoms of lobar pneumonia ascribed to toxemia.

ON THE TOXIC SUBSTANCES FROM VIRULENT PNEU-MOCOCCI AND PNEUMONIC LUNGS AND THE INFLUENCE THEREON OF QUININ AND UREA HYDROCHLORID, ETHYLHYDROCUPREIN HYDROCHLORID, AND OTHER CINCHONA DERIVATIVES

STUDIES IN PNEUMONIA, VI

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INTRODUCTION

In clinical studies of the beneficial influence of quinin salts and allied compounds in the pneumonias of man, three facts stand out: (1) The relief to toxic symptoms without appreciable influence on the evolution of physical signs, (2) the change of termination from crisis to lysis, and (3) the absence of cinchona poisoning, even when enormous doses are given. (This does not apply to ethylhydrocuprein, however). There is nothing in the known pharmacologic influence of quinin as a function modifier that will account for either the first or the second of these facts. Nor can bactericidal action alone explain them. The third fact indicates the presence of a quinin-tolerance in the subjects of pneumonia.

Hence the hypothesis has been put forth of a possible neutralizing influence of the drug; i. e., a mutual antagonization or inhibition, physical or chemical, direct or indirect, of quinin by pneumonia poison-complex, and of pneumonia poison-complex by quinin.[1]

The term pneumonia poison-complex rather than pneumotoxin, has been used, because it is not accurately known just what are the poisons present in pneumonia—whether they are all of microbic origin directly or indirectly, or whether they are produced in part or in whole by the human organism. The main facts being unknown, the details as to site of production, manner of distribution, chemical and biologic nature, action, etc., can only be conjectured; and no opinion can be

[1] Solis-Cohen, S.: N. Y. Med. Jour., 1916, 103, p. 1065.

hazarded as to whether the poisons belong wholly to offensive reactions or in part to defensive ones.

To test the hypothesis of the special relation of pneumonia poisons to quinin, therefore, involves much groping in the dark, in the process of establishing or eliminating various possibilities; and an endeavor to find the poison or poisons in question should logically precede such investigations.

It has been deemed practicable, however, to pursue the two lines of research together, partly in the hope that one might throw light on the other; and partly that at least certain preliminary questions as to the possible action of quinin might be determined.

THE PNEUMOTOXIN

In the attempt to isolate the substance responsible for the toxic symptoms in the pneumonias of man, investigators have focussed their attention on the pneumococcus. Neufeld and Dold[2] showed that by the action in vitro of complement on the precipitate resulting from the addition of pneumococci to the corresponding immune serum, a toxic substance is produced which, on primary intravenous injection into guinea-pigs produces anaphylactic-like symptoms and death. They concluded that this anaphylatoxin of Friedberger[3] is set free by the body fluids and that pneumonia is a "mild and protracted form of anaphylaxis." Vaughan and Wheeler[4] give a similar explanation, based on their studies of protein products. Rosenow[5] thinks that the autolytic products of the pneumococcus which he demonstrated in vitro in the absence of serum, and which are similar in their properties to the anaphylatoxin of Friedberger, are liberated in vivo by the ferments in the bacterial cell. But as pointed out by Cole, who gives a complete review of the literature,[6] the symptoms produced by the injection of anaphylatoxin or bacterial extract resemble very remotely those presented during a pneumococcus infection. Furthermore, Cole found that even when autolysis of the pneumococci is prevented by dissolving large masses of them in sodium choleate, a highly toxic and hemolytic substance is liberated which produces anaphylactic symptoms and death on primary injection into guinea-pigs. He concludes that the pneumococcus poison exists preformed in the bacterial cell

[2] Dold, H.: Das Bakterienanaphylatoxin, 1912.
[3] Ztschr. f. Immunitätsforsch., 1909, 2, p. 208; 3, p. 692; 1910, 4, p. 636.
[4] Jour.. Infect. Dis., 1907, p. 476.
[5] Ibid., 1911, 9, p. 190; 1912, 10, p. 113.
[6] Jour. Exper. Med., 1912, 16, p. 644.

and that its liberation depends not on the action of its own autolytic ferments, but on the dissolution of the pneumococcus by the body fluids.

What part this endocellular pneumococcus toxin plays in the symptom-complex and changes in pneumonia is a matter of conjecture. It is possible that its continuous liberation in vivo (presumably accomplished with facility by the action of the tissue enzymes: trypsin, leukoproteases, etc.) may result in the initial toxemia manifest in general disturbances such as cardiac, respiratory and vasomotor symptoms. The pneumotoxin, moreover, increases the permeability of the walls of the capillaries lining the alveoli for the various blood proteins, erythrocytes, leukocytes, fibrin and enzymes. Friedrich Müller[7] has shown that the enzymes in the exudate are introduced from without and are foreign to the lung tissue. These normal constituents of the circulating body fluids, when transferred to and accumulated in the alveolar spaces form the exudate. We have shown elsewhere[8] that the pneumonic exudate is composed of normal serum proteins. The extreme leukocytosis in pneumonia favors this phenomenon.

With the body fluids depleted of their normal proteins, there is a diminution in their osmotic pressure, as shown by Sandelowsky,[9] Achard, Tourraine and Saint Girons,[10] Epstein,[11] and Rowe.[12] To compensate for this there is the retention of chlorids, long clinically known and recently studied by Rowntree,[13] Medigreceanu[14] and Peabody,[15] which keeps the osmotic pressure constant.

It may be further assumed that pneumococci proliferating in the body fluids liberate the proferments that initiate the autolysis of the fibrin, leukocytes, etc., in the lung. Taylor[16] points out that bacterial extracts and tissue juices activate proferments such as propepsin. The toxic products, however, tend to inhibit this autolysis with the production of only partly digested products such as albumoses, proteoses and peptones, as observed by Turro,[17] Matthes,[18] Levaditi,[19] Pouchet,[20] and

[7] Verhandlung der Naturforschenden Gesellschaft in Basel, 1902, 13.
[8] Weiss, Charles, et al.: The Mechanism of the crisis in pneumonia (to be published).
[9] Deutsch. Arch. f. klin. Med., 1909, 96, p. 455; 1910, 100, p. 324.
[10] Arch. de Med. Exper. et d'anat. Path., 1912, 24, p. 647.
[11] Am. Jour. Med. Sc., 1917, 154, p. 638.
[12] Arch. Int. Med., 1917, 18, p. 455.
[13] Bull. Johns Hopkins Hosp., 1908, 19, p. 367.
[14] Jour. Exper. Med., 1911, 14, p. 289.
[15] Jour. Exper. Med., 1913, 17, p. 71.
[16] Digestion and Metabolism, 1912.
[17] Centralbl. f. Bakteriol., 1912, 32, p. 105.
[18] Arch. f. exp. Path. u. Pharm., 1895, 36, p. 437.
[19] Ann. de l'Inst. Pasteur, 1903, 17, p. 187.
[20] 1883, 96, p. 1506 and 1601.

Auld.[21] These products are toxic. They aid in further inhibition of autolysis and when absorbed, increase the coagulation time of the blood, as shown by Dochez,[22] Hayem,[23] Pye-Smith,[24] and Coleman.[25] Opie[26] has furthermore shown that the serum of inflammatory exudates retards the action of the autolytic enzymes within the leukocytes.

The period of accumulation of greatest amount of pneumotoxin and hence of toxic albumoses, proteoses, etc., is the period of the crisis. As the pneumococci continue to proliferate, the increasing amount of acid (as pointed out by Netter, Bougault and Salanier,[27] and of bactericidal substances in the exudate (observed by Conradi[28]) combined with the phagocytic action of the leukocytes (Lamar and Meltzer,[29] Tschistovitch,[30] Winternitz and Hirschfelder,[31] Winternitz and Kline[32]), and the production of various antibodies (observed by Clough,[33] Dochez,[34] Jobling,[35] Rosenow,[5] Cole,[36] Bull[37] and others) checks their growth and toxin production. That antibodies may be formed to the toxin itself in addition to other antibodies, is suggested by the increase of the globulins of the serum (Rowe[12] and Epstein[38]), a phenomenon which is generally found to be associated with antibody formation, as shown by Hurwitz and Meyer,[39] Banzhaf and Gibson,[40] Gibson and Collins[41] and others.

Moreover, the continuous action of the toxins, aside from antibody production, changes the primary inhibitory effect on autolysis into an accelerating one. This is suggested by the work of Hess and

[21] Selected Researches in Pathology, 1901.
[22] Jour. Exper. Med., 1912, 16, p. 693.
[23] Du sang et de ses alterations anatomiques, 1889.
[24] Allbut and Rolleston, System of Medicine, 1898, 5, p. 91.
[25] Biochem. Jour., 1907, 2, p. 184.
[26] Jour. Exper. Med., 1905, 7, p. 316.
[27] Compt. rend Soc. de Biol., 1917, p. 80, and 97.
[28] Beitr. z. Chem. Physiol. u. Path., 1901, I, p. 193.
[29] Jour. Exper. Med., 1912, 15, p. 133.
[30] Ann. de l'Inst. Pasteur, 1890, 4, p. 285; 1904, 18, p. 304.
[31] Jour. Exper. Med., 1913, 17, p. 657.
[32] Ibid., 1915, 21, p. 320.
[33] Bull. Johns Hopkins Hosp., 1913, 24, p. 295.
[34] Jour. Exper. Med., 1912, 16, p. 665.
[35] Ibid., 1915, 22, p. 568.
[36] Ibid., 1914, 20, p. 346.
[37] Ibid., 1915, 22, p. 457.; 1916, 24, p. 7.
[38] Ibid., 1912, 16, p. 719; 1913, 17, p. 444; 1914, 20, p. 334.
[39] Ibid., 1916, 24, p. 515.
[40] Ibid., 1910, 12, p. 3.   Gibson and Famulaner, L. W.: Collected Studies, Bureau of Laboratories, Dept. of Health, City of New York, 1915, 8, p. 208.
[41] Jour. Biol. Chem., 1907, 3, p. 232.

Saxl.[42] This accelerated autolysis in turn hastens antibody production as pointed out by Blum.[43] The products of autolysis are now completely digested proteins in the amino-acid stage which are nontoxic and easily absorbed and eliminated.

The transition from inhibitory to accelerating action of pneumotoxin on autolysis is governed by the laws of mass action. Hence the change from febrile toxemia to the afebrile atoxic state is necessarily an abrupt one — crises. The period is marked by the sudden drop in temperature and relief from toxemic symptoms and return to normal coagulation time of the blood. It is clinically a period of peril, and may terminate in death—a fact not to be lost sight of in analysis or in experimental study. If successfully passed, resolution sets in, with removal (autolysis) of exudate.

The autolysis is probably accomplished by the normal proteolytic enzymes of the body. Hartman[44] suggests that the fibrin requires specific antibodies for its removal, and Ascoli[15] is of the opinion that isolysins are formed to remove the cellular constituents. He demonstrated such isolysins after the crisis.

That the pneumotoxin exerts no influence on the lung tissue itself and that the latter plays no part in the formation of the exudate is well known, as mentioned by Wells.[46] Our own observations[8] based on various anaphylactic and chemical studies substantiate this.

Views similar to this have been expressed by Hedin,[47] Opie[26] Jobling and Peterson,[35] Dick,[48] Hektoen,[49] and Falls.[50] At present we are engaged in subjecting the various phases of our hypothesis to experimentation.

The opinion that the exudate plays an important and specific rôle in lobar pneumonia is indicated by the work of Rosenow and Arkin[51] who showed that extracts of pneumonic lungs injected intravenously into dogs delay the coagulation time of the blood, and produce symptoms simulating anaphylaxis. Our own work on the comparative toxicity of normal and pneumonic lungs described elsewhere[52] demon-

[42] Wiener klin. Wchschr., 1908, 21, p. 248 and 486.
[43] Beitr. z. chem. Physiol. u. Path., 1904, 5, p. 142.
[44] Jour. Infect. Dis., 1913, 13, p. 69 and 499.
[45] München. med. Wchnschr., 1901, 48, p. 1241.
[46] Chemical Pathology, 1914.
[47] Ztschr. f. physiol. Chem., 1907, 52, p. 412.
[48] Jour. Infect. Dis., 1912, 10, p. 383.
[49] Jour. Am. Med. Assoc., 1914, 62, p. 254.
[50] Jour. Infect. Dis., 1915, 16, p. 466.
[51] Jour. Infect. Dis., 1912, 11, p. 480.
[52] Kolmer, J. A., Weiss, C., and Steinfield, E.: Jour. Infect. Dis., 1918, 22, p. 469.

strates the toxicity of the pneumococcus exudate. We have, further-more, obtained specific anaphylactic reactions in guinea-pigs sensitized to such extracts. We shall report on this and other corroborative evidence in another paper.[8]

These two substances, the pneumococcus hemotoxin and the toxic extract of a pneumonic lung, seemed to afford a point of attack in the problem of the action of quinin on pneumococcic poisons in general, and the observations which are set forth in detail in this paper were made to determine whether any neutralizing action could be demon-strated.

I. Is There a Neutralizing Power of Quinin and Its Congeners on Pneumotoxin?

Technic of Preparation of Pneumotoxin.—The demonstration of an endo-cellular toxin in the pneumococcus is fraught with many difficulties. The virulence of the organism, the mediums in which it is grown, the temperature and time of incubation, the amount of autolysis, the presence of traces of broth proteins, the thickness of the emulsion of pneumococci, the volume of choleate used, and a host of other details determine the success or failure of the prepa-ration of the pneumococcus toxin. In spite of the fact that we followed in detail the precautions mentioned by Cole, we were unable to produce uniformly a toxin which was hemolytic and also produced anaphylaxis on primary intra-venous injection in guinea-pigs. Our results with rats were almost uniformly negative, probably due to the extremely high tolerance of the rat for anaphyla-toxins. This subject has been recently studied by Novy and his associates.[53]

The organism used was pneumococcus Type I obtained from Dr. Rufus Cole. It was grown in tubes of beef infusion broth containing 0.1% glucose and 1% Witte's peptone to which was added a drop of freshly defibrinated human, horse or rabbit blood. The reaction of the medium was slightly alkaline to phenol-phthalein.

It was found advisable, in order to obtain a luxuriant and virulent culture, to transplant every other day, and from time to time, to pass the organism through a mouse. In this way the M. L. D. for mice (24 hours) was kept constantly at 0.000,000,1 c c of a 24-hour broth culture.

The technic employed was briefly as follows: 1,000 to 1,500 c c of a 24-hour broth culture was centrifugalized for 30-60 minutes at high speed. The bacterial sediment was washed once in salt solution (0.85%) and after a second centri-fugalization the supernatant fluid was completely removed. To the sediment were added 5 c c of salt solution and 5 c c of a mixture of 2% solutions of pri-mary and secondary sodium phosphate (1:8). To the thick emulsion thus obtained was added 1-2 c c of a 2% solution of sodium choleate (in normal salt solution). This mixture was placed in a 37 C. water-bath for one-half hour and frequently stirred; the total volume was now made up to 75 or 100 c c with salt solution and the resulting mixture kept in the refrigerator until used.

A control fluid containing sodium choleate and primary and secondary sodium phosphate was prepared in exactly the same way. The volume of pneumococcus emulsion was of course replaced by salt solution.

[53] Novy, DeKruif, et al.: Jour. Infect. Dis., 1917, 20, p. 498.

The Toxicity of Pneumotoxin by Animal Injection.—The toxicity of the pneumotoxin was tested by intravenous injection in guinea-pigs and white rats and by intraperitoneal injection in white mice. When injecting pneumotoxin intraperitoneally into mice, it became extremely important to culture the fluid for purpose of ascertaining whether or not any pneumococci had escaped solution in the sodium choleate, and also to culture the heart's blood of all mice dying as a result of injection. The results are given in Tables 1 and 2.

TABLE 1

TOXICITY OF PNEUMOTOXIN BY INTRAVENOUS INJECTION IN GUINEA-PIGS *

Pneumo-toxin No.	Weight of Animal in Grams	Dose Injected in C c	Results
1	350	6	Immediate anaphylactic death
1	400	6	Anaphylactic symptoms with death in 24 hrs.
1	320	6	Anaphylactic symptoms with death in 24 hrs.
Control fluid 1	315	6	No symptoms
2	210	2	Died. 24 hours
2	205	2	Died. 24 hours
2	200	2	Died. 48 hours
2	205	5	Died. 24 hours
2	200	2	Died. 72 hours
Control fluid 2	180	2	Died. 24 hours
Control fluid 2	205	3	Died. 24 hours
9	265	8.5	Dyspnea; died in 5 days
9	270	5.5	Slight toxic symptoms; recovered
10	310	6.5	Severe dyspnea; recovered
10	300	5.5	Moderate shock
10	255	4.5	Mild dyspnea; recovered

* All animals were observed for a period of one week.

TABLE 2

TOXICITY OF PNEUMOTOXIN BY INTRAPERITONEAL INJECTION

Mice				Rats			
Pneumo-toxin No.	Weight of Animal in Grams	Dose per 100 Grams	Results	Pneumo-toxin No.	Weight of Animal in Grams	Dose per 100 Grams	Results
4	19	1.0	Died, 24 hours	6	84	5.0	Dyspnea; recovered
4	20	0.5	Died, 24 hours	6	81	4.0	No symptoms
4	20	0.06	Died, 24 hours	6	82	2.5	No symptoms
Control fluid 4	20	1.0	Survived	6	75	2.0	No symptoms
				Control fluid 6	70	4.0	No symptoms

The Neutralizing Power of Quinin and Its Compounds on Pneumotoxin in Vivo.—After having thus determined the lethal dose of each preparation of pneumotoxin, we attempted to neutralize its toxicity by means of the various cinchona derivatives, using the following methods:

1. In Vitro: The toxin and drug were mixed in the test tube in proper proportions and incubated either at room temperature or at 37 C. for 1, 2 or 3 hours and injected. The in vitro method had to be abandoned for it was observed that sodium choleate, protein solutions and colloidal suspensions (such as agar,

saponin and gum mastic) as well as serum will precipitate quinin. The latter substance is mentioned by MacGilchrist.[54]

2. In Vivo: The proper amount of drug was injected into the animal 2 or more hours before the injection of the toxin.

The results are summarized in Tables 3 and 4.

TABLE 3

Neutralizing Power of Cinchona Derivatives for the Toxicity of Pneumotoxin

Weight of Animal in Grams	Pneumotoxin		Quinin Salt	Dose of Quinin per 60 Kilo	Results
	No.	Dose			
185	2	5 c c	Ethylhydrocuprein hydrochlorid	1.5	Anaphylactic; death 5 minutes
195	2	5 c c	Quinin and urea hydrochlorid	1.5	Died 24 hours
195	Control	0	Ethylhydrocuprein hydrochlorid	1.5	No symptoms
195	Control	0	Quinin and urea hydrochlorid	1.5	No symptoms

Pneumotoxin and quinin salt were incubated at room temperature for one hour and injected intravenously into guinea-pigs.

TABLE 4

Neutralizing Power of Cinchona Derivatives for the Toxicity of Pneumotoxin

Weight in Grams	Dose of Cinchona		Results
	Name of Salt	Grams per 60 Kilo	
In Vitro			
18	Ethylhydrocuprein hydrochlorid	2.0	Died, 24 hours
16	Ethylhydrocuprein hydrochlorid	1.0	Died, 24 hours
19	Quinin and urea hydrochlorid	2.0	Died, 24 hours
21	Quinin and urea hydrochlorid	1.0	Died, 24 hours
In Vivo			
16	Ethylhydrocuprein hydrochlorid	1.0	Died, 24 hours
20	Ethylhydrocuprein hydrochlorid	0.5	Died, 24 hours
18	Quinin and urea hydrochlorid	1.0	Died, 24 hours
18	Quinin and urea hydrochlorid	0.5	Died, 24 hours

In Vitro Tests: 2 M.L.D. of pneumotoxin were incubated at 37 c c for 2 hours with the proper dose of quinin and injected intraperitoneally into mice. In Vivo Tests: The proper dose of quinin was injected intraperitoneally and 2 M.L.D. of the pneumotoxin were given in the same way. Controls: Receiving the doses of quinin given in the tables survived; those receiving the pneumotoxin alone died.

As shown in the tables, the cinchona derivatives do not exert any gross neutralizing action on the toxicity of pneumococcus toxin. In vivo experiments conducted with guinea-pigs gave negative or inconstant results. It was thought highly desirable to study the effect of the repeated injection of sublethal doses of the pneumotoxin followed by

[54] Scient. Mem. Off. Med. and Sanit. Depts., India, 1911, No. 41.

similar doses of the cinchona derivatives. This slower method of absorption approaches more closely the condition in human pneumonia. But because of the difficulty of preparing the pneumotoxin and of its extreme lability and rapid deterioration this procedure was found impracticable.

Hemolytic Activity of Pneumotoxin.—Cole[36] showed that the toxin liberated by the dissolution of washed pneumococci in sodium choleate was hemolytic for red blood cells. He found that various normal serums, solutions of egg albumin, cholesterin, lecithin, and trypsin will destroy this hemolytic power; that the inhibitory power of serum of pneumonic patients was no greater than of normal serum.

We attempted to demonstrate any neutralizing action of various quinin salts in relation to the hemolytic action of the pneumotoxin, in the hope that it would throw some light on the influence of the cinchona derivatives in the treatment of pneumonia.

Technic of Hemolytic Tests.—Fresh guinea-pig corpuscles washed free from serum were used in a 1% suspension in salt solution, and in constant dose of 1 c c.

Toxin or poison was made up with salt solution into proper dilution and pipetted in doses ranging from 0.1-2 c c.

The total volume in each tube was 3 c c.

Incubation was effected either in a 37 C. water bath for 1 hour or in a dry incubator at 37 C. for 2 hours.

Readings were made at the end of the period of incubation and again after the cells have settled down in the refrigerator over night.

The results obtained are illustrated in Table 5.

TABLE 5

THE HEMOLYTIC ACTIVITY OF PNEUMOTOXIN

Substance	Dose of Pneumotoxin C e						
	0.1	0.3	0.5	0.7	1.0	1.5	2.0
Pneumotoxin 1	NH	NH	NH	NH	NH	NH	NH
Control fluid 1	NH	NH	NH	NH	NH	NH	NH
Pneumotoxin 2	VSH	SH	SH	MH	MH	CH	CH
Control fluid 2	NH	NH	NH	NH	NH	SH	CH
Pneumotoxin 3	NH	NH	CH	CH	CH	CH	CH
Control fluid 3	NH	NH	NH	NH	NH	CH	CH
Pneumotoxin 3*	VSH	VSH	CH	CH	CH	CH	CH
Control fluid 3	NH	NH	NH	NH	CH	CH	CH
Pneumotoxin 4	NH	NH	NH	NH	NH	NH	NH
Control fluid 4	NH	NH	NH	NH	NH	NH	NH
Pneumotoxin 5	NH	NH	NH	SH	MH	CH	CH
Control fluid 5	NH	NH	NH	SH	CH	CH	CH
Pneumotoxin 6	NH	NH	SH	MH	CH	CH	CH
Control fluid 6	NH	NH	SH	CH	CH	CH	CH
Pneumotoxin 7	MH	CH	CH	CH	CH	CH	CH
Control fluid 7	NH	NH	NH	MH	CH	CH	CH
Pneumotoxin 9	CH	CH	CH	CH	CH	CH	CH
Control fluid 9	NH	NH	SH	MH	CH	CH	CH
Pneumotoxin 10	NH	NH	NH	VMH	CH	CH	CH
Control fluid 10	NH	NH	SH	MH	CH	CH	CH

NH = no hemolysis; VSH = very slight hemolysis; SH = slight hemolysis; MH = marked hemolysis; VMH = very marked hemolysis; CH = complete hemolysis.

* This pneumotoxin was retested after one week's storage in the refrigerator.

As shown in Table 5, four of the pneumotoxins (2, 3, 7, and 9) showed a more marked hemolytic activity for guinea-pig cells than the control fluids. Cole points out that the hemolytic and toxic properties of a solution of pneumococci in sodium choleate are not always concomitant. Our findings confirm this opinion. From the results of our attempts at preparing pneumotoxin we are led to believe that the glucose in the culture medium, while essential for the production of the hemolytic properties, interferes with the liberation of the toxic constituents by producing cholic acid from the sodium choleate (Table 6).

TABLE 6

Summary of the Toxic and Hemolytic Properties of Various Preparations of Pneumotoxin

Pneumo- toxin No.	Volume of Broth in C C	Per Cent. of Glucose in Broth	Degree of Toxicity	Hemolytic Activity
1	1000	0.1	+++	None
2	1500	0.1	++	++
3	1000	0.1	0	+++
4	2000	0.1	+	None
5	1500	None	+	±
6	1500	None	+	±
7	1500	0.03	0	+++
9	1400	0.1	+	+++
10	1400	None	+	+

+++ Very marked hemolytic activity or toxicity.
 ++ marked hemolytic activity or toxicity.
  + slight hemolytic activity or toxicity.
  ± doubtful (hemolytic activity no greater than control fluid).
  0 = not determined.

The Neutralizing Power of Cinchona Derivatives for the Hemotoxin of Pneumococci in Vitro.—In the course of our studies on the neutralizing power of the cinchona derivatives for the hemotoxin of pneumococci, we observed that the solutions of quinin used (1% in salt solution) were of themselves very hemolytic. This hemolytic activity was partly due to the free acid present in the various soluble salts of quinin. It was found impossible to neutralize this acidity, since the quinin would precipitate on the addition of alkali. The following technic was therefore adopted: The hemolytic activity of the various quinin salts in 0.1% and 1% solutions, of the toxin and of the control fluid was titrated for guinea-pig cells. In one series of test-tubes were placed the largest nonhemolytic doses of the various quinin salts; in a second series the smallest hemolytic dose, in a third gradually increasing doses. To each tube of Series 1 and 2 were added increasing doses of toxin. Each tube of Series 3 received 3 M. H. D. of pneumotoxin. The same procedure was repeated using the control fluid. We found that the quinin salts exerted no inhibition on the hemolytic activity of pneumotoxin. In fact the reverse phenomenon, a summation of hemolytic powers was observed.

II. The Influence of Cinchona Derivatives on the Toxic Substances Obtainable from Pneumonic Lungs

Preparation of Lung Extracts.—A report of the method of preparation, the comparative toxicity and biologic specificity of human pneumonic lung extracts is given in another communication.[52] We wish here briefly to indicate the technic and the results of attempts to neutralize the toxic substance in such extracts: A pneumonic lung in the stage of gray hepatization was obtained as soon as

possible after necropsy; smears were made from various sections and stained for pneumococci by Gram's stain. The lung tissue was passed through a meat grinder, ground up in a mortar with washed quartz sand and finally squeezed in a Buchner press with a force of 250 kg. per square centimeter. The juice thus obtained was centrifuged, filtered through paper and preserved in the refrigerator with 0.5% phenol. Injections were made in guinea-pigs, mice and rabbits by intraperitoneal and intravenous routes.

We found that the exudate of human pneumonic lung is highly toxic, producing anaphylactic-like symptoms in guinea-pigs and rabbits and paralysis on repeated intramuscular injection in the latter. This last observation suggests the presence of a toxic albumose such as demonstrated by Auld[21] in the tissues of rabbits dying of pneumonic infections.

Summary of the Neutralizing Power of Cinchona Derivatives for the Toxicity of Pneumonic Lung Extracts.—Using both in vitro and in vivo methods it was found that quinin hydrobromid exerted a very marked neutralizing action on the toxicity of pneumonic lung extracts in that therapeutic doses prolonged the life of the animal up to five days or more after the injection of 1 M.L.D. of the extract; quinin hydrochlorid, quinin and urea hydrochlorid and ethylhydrocuprein hydrochlorid afforded little or no protection. The results are shown in Tables 7, 8, and 9.

TABLE 7

The Neutralizing Power of Various Cinchona Derivatives on the Toxicity of Pneumonic Exudates in Guinea-Pigs

Weight of Animal in Grams	Cinchona Compound	Results
175	Ethylhydrocuprein hydrochlorid....................	Died at once
195	Quinin and urea hydrochlorid......................	Died in 30 minutes
215	Quinin bisulphate................................	Died in 6 days
200	Quinin hydrochlorid..............................	Died in 2 days
215	Hydroquinin hydrochlorid.........................	Died at once
210	Quinin hydrobromid..............................	Died in 5 days

One M.L.D. of pneumonic lung extract was incubated for 2 hours at 37 C. with a dose of cinchona equivalent to 1 gm. per kilo of body weight and injected intravenously.

TABLE 8

The Neutralizing Power of Various Cinchona Derivatives on the Toxicity of Pneumonic Exudates in Mice

Weight in Grams	Cinchona Derivative	Grams of Drug per 60 Kilo	Results
24	Ethylhydrocuprein hydrochlorid.............	2.0	Died, 24 hours
21	Ethylhydrocuprein hydrochlorid.............	1.0	Died, 24 hours
17	Quinin and urea hydrochlorid...............	2.0	Died, 24 hours
17	Quinin and urea hydrochlorid...............	1.0	Died, 24 hours
14	Quinin bisulphate...........................	2.0	Died, 24 hours
18	Quinin bisulphate...........................	1.0	Died, 24 hours

Mice were injected intraperitoneally with 32 M.L.D. of the lung poison 2 hours after the administration of the drug. Controls receiving the drugs alone survived. Controls receiving pneumonic lung extract alone died in 24 hours.

TABLE 9

Neutralizing Power of Cinchona Derivatives for the Toxicity of Pneumonic
Exudate in Rabbits

Weight of Animal	Cinchona Derivative	Dose of Cinchona in Grams per 60 Kilo	Results
1280	Ethylhydrocuprein hydrochlorid...........	0.5	Died in 24 hours
1460	Ethylhydrocuprein hydrochlorid...........	0.2	Died in 24 hours
1300	Quinin and urea hydrochlorid.............	0.5	Died in 24 hours
1040	Quinin and urea hydrochlorid.............	0.2	Died in 24 hours
750	Quinin hydrobromid......................	0.5	Died in 24 hours
1200	Quinin hydrobromid......................	0.2	Survived

Rabbits were injected into the muscle of the leg—quinin and toxin being given simultaneously in opposite legs. The dose of poison was uniformly 1 c c per kilo. Controls receiving the doses of quinin alone survived.

Hemolytic Activity of Extracts of Human Pneumonic Lungs.—Using the technic described under the heading—pneumotoxin—it was found that the pneumonic lung extracts were markedly hemolytic for guinea-pig erythrocytes, the M.H.D. being in one case 0.3 c c of a 1 : 100 dilution. Control experiments with normal (nonpneumonic) lungs were very slightly lytic in 1 : 50 dilution.

Summary of Antihemolytic Activity of Quinin Salts.—The results of the studies on the inhibitory effect of the cinchona derivatives on the hemolytic activity of pneumonic exudates were very difficult of interpretation. Using dilutions of these salts in doses ranging from 0.00001 to 0.002 gm. and 1 M.H.D. of poison we found that ethylhydrocuprein hydrochlorid, quinin, and urea hydrochlorid, gave a slight inhibition when large doses (of themselves hemolytic) were used. Quinin hydrochlorid, quinin hydrobromid, quinin bisulphate, and hydroquinin hydrochlorid behaved in a reverse way, giving marked inhibition in nonlytic doses and no inhibition in lytic doses. The ease of precipitation of the former salts in the presence of protein solutions and the greater solubility of the latter in protein solutions are factors entering into an explanation of this phenomenon. Sherwood[55] has recently studied this subject.

The Occurrence of Toxic Substances in the Blood of Pneumonic Patients.— Attempts to detect toxic substances in the blood of pneumonic patients have been made by numerous workers. Cole[6] has shown that the filtered active serum of a rabbit dying of a very severe pneumococcic septicemia does not contain any demonstrable toxins. Knowing that the Berkefeld filter retains toxic albumins, complement and various large protein molecules[56, 57] we repeated this experiment, using the unfiltered serum but were unable to produce anaphylactic symptoms in a rabbit. Rosenow,[5] on the other hand, has obtained anaphylactic reactions by the primary injection of pneumonic serum which was allowed to autolyze at ice-box temperature for one week.

Since pneumococcic septicemia in rabbits and mice differs greatly from lobar pneumonia in man, we thought it might be possible to detect in human blood toxic substances derived either from the autolysis of the pneumococci or the leukocytes, or more particularly from the diffusion of toxic protooses

[55] Jour. Infect. Dis., 1917, 20, p. 185.
[56] Vaughan, V. C.: Protein split products in relation to immunity and disease, 1913.
[57] Madsen, Th., and Noguchi, H.: Jour. Exper. Med., 1907, 9, p. 18.

and albumoses into the circulation. With this object in view, we injected the whole blood, defibrinated blood, red blood cells, leukocytes and serum of normal persons and of patients suffering with lobar pneumonia—often moribund or at the height of the disease. These experiments were done on rabbits, rats and mice by both intravenous and intraperitoneal routes. Using graded doses calculated per body weight of the animal, we were unable to detect any difference in the toxicity of normal and pneumonic blood. Table 1 shows our results with active serums. The normal serum (taken from a healthy medical student) seems to be more toxic than the pneumonic. The pneumonic blood (Table 1) was taken from a patient with a temperature of 102 F., pulse 128 to 140, respiration 40 to 65; consolidation of lower right lobe; increased tactile fremitus, vocal resonance, bronchial breathing; no quinin was given.

TABLE 10

Comparative Toxicity for Mice of Normal Pneumonic Serums

Normal			Pneumonic		
Weight in Grams	Dose in C C per Kilo	Results	Weight in Grams	Dose in C C per Kilo	Results
16	1	Survived	16	1	Survived
20	2	Died, 48 hours	18	2	Survived
19	6	Died, 48 hours	14	6	Survived
15	10	Died, 72 hours	17	10	Survived
16	12	Survived	13	15	Survived
15	15	Died, 48 hours	18	20	Survived
20	20	Died, 48 hours	23	50	Survived
17	50	Survived			

Mice were injected intraperitoneally with the sterile, active serum in proper dilution.

THE INCREASED TOLERANCE FOR QUININ IN PNEUMONIA

To begin an attack on the problem from another angle, a number of observations were made to determine whether or not the pneumococcus protein or substances present in the blood of pneumonia patients would exert any neutralizing influence in relation to cinchona poisoning.

The method consisted of mixing lethal doses of various quinin salts with nontoxic doses of pneumococcus protein or of pneumonic blood fractions (red blood cells, leukocytes, defibrinated blood, etc.) and incubating for one hour or more at 37 C. As pneumococcus protein, the washed and killed pneumococci obtained by centrifuging 500 c c of a 48-hour broth culture were used suspended in 20 c c salt solution. The mixtures were injected intraperitoneally into mice and rats. Several of the tests were done by injecting the dose of cinchona 3 or 4 hours after the administration of the pneumonic substance.

The work was repeated several times, but our results were uniformly negative with this method.

TABLE 11

The Neutralizing Power of Pneumococcic Protein and of Pneumonic Serum for the Toxicity of Cinchona Derivatives

Weight in Grams	Substance	Dose in C C per Kilo	Cinchona Salt	Dose of Drug in Grams per Kilo	Results
12	Pneumococcus protein	10	Ethylhydrocuprein hydrochlorid	0.7	Died, 24 hours
17	Pneumococcus protein	10	Ethylhydrocuprein hydrochlorid	0.5	Died, 24 hours
17	Pneumococcus protein	10	Quinin and urea hydrochlorid	0.7	Died, 24 hours
23	Pneumococcus protein	10	Quinin and urea hydrochlorid	0.5	Died, 24 hours
20	Pneumonic serum	100	Quinin and urea hydrochlorid	0.5	Died, 10 minutes
15	Pneumonic serum	50	Quinin and urea hydrochlorid	0.5	Died, 10 minutes
14	Pneumonic serum	10	Quinin and urea hydrochlorid	0.5	Died, 10 minutes

Emulsion of dead pneumococci mixed with dose of drug was incubated at 37 C. for 3 hours and injected intraperitoneally into mice. Controls receiving emulsion alone survived. Pneumonic serum, sterile and active, was injected intraperitoneally 4 hours before the administration of the drug.

DISCUSSION

While our results on the study of the influence of quinin and urea hydrochlorid, ethylhydrocuprein hydrochlorid, and allied cinchona derivatives on the toxic substances obtainable from virulent pneumococci and pneumonic lungs have been largely negative, the question as to the rôle of quinin in the treatment of pneumonia is still to be solved. As to the particular problems here reported on, more refined methods of study are to be devised. The action, if any, is not of a gross physical or chemical order. Suggestive are the exceptional action of quinin hydrobromid in combating the toxicity of pneumonic lung exudate, and the somewhat paradoxical results of various cinchona derivatives in antagonizing the hemolytic action of the same substance. Further research in many directions is here indicated. It may be added parenthetically that quinin hydrobromid and quinin dehydrobromid have proved of considerable clinical usefulness in the treatment of lobar pneumonia in man—a usefulness especially marked in Type II infections. The high pneumococcidal value of these salts was set forth in our first study.[58] We have shown elsewhere that quinin exerts a highly specific and marked pneumococcidal action both in vitro and vivo.[58] We have demonstrated its power of enhancing phagocytosis.[59] Its

[58] Solis-Cohen, S., Kolmer, J. A., and Heist, G. D.: Jour. Infect. Dis., 1917, 20, p. 40.
[59] Ibid., p. 101.

property of reducing the plane of nitrogen metabolism described by von Boeck[60] and its general influence on antibody production may be the factors involved. We are at present studying the latter problem.

SUMMARY

Pneumococci when dissolved in sodium choleate liberate a pre-formed endocellular toxin which is lytic for erythrocytes. Our work confirms the observations of Cole.

The liberation of this toxin in vitro is a most inconstant phenomenon and is fraught with many technical difficulties.

Different preparations of the pneumotoxin vary considerably in their toxicity for guinea-pigs, mice and rats. Anaphylactic shock followed by immediate or delayed death, very severe dyspnea with recovery or only mild respiratory disturbances with death in 24 hours are observed after the intravenous injection into guinea-pigs (200-300 gm. in weight) of doses varying from 9-2 c c of the pneumotoxin.

Rats are highly resistant to the pneumotoxin. This is in accord with the findings of Novy.

The hemolytic power of the pneumotoxin was studied, using guinea-pig erythrocytes. It was observed to be inconstant and not always concomitant with the degree of toxicity.

Quinin and urea hydrochlorid, ethylhydrocuprein hydrochlorid, and other derivatives of cinchona were found to exert no influence on either the toxicity or the hemolytic activity of pneumotoxin.

Extracts of pneumonic lungs in the stage of gray hepatization were prepared and found to be extremely toxic for guinea-pigs and rabbits on intravenous and intramuscular injection. Convulsions, dyspnea and immediate death followed the intravenous injection of guinea-pigs and rabbits with doses of 0.7 c c and 0.2 c c, respectively, per kilo of body weight.

Therapeutic doses of quinin bisulphate, quinin hydrochlorid and particularly quinin hydrobromid prolonged to a recognizable extent the lives of animals receiving 1 M.L.D. of pneumonic lung extract.

These pneumonic lung extracts were found to be markedly lytic for erythrocytes, the M.H.D. being about 0.3 c c of a 1 : 100 dilution with the technic employed.

Quinin and urea hydrochlorid and ethylhydrocuprein hydrochlorid had slight inhibitory effect on the hemolytic activity of the lung

[60] Untersuchungen über die Zersetzung des Eiweisses im Thierkörper unter dem Einflusse von Morphin, Chinin und Arsenige Säure, 1871.

extracts when large, hemolytic doses of the drugs were used. Quinin hydrochlorid, quinin hydrobromid, quinin bisulphate, and hydroquinin hydrochlorid behaved in a reverse way, exhibiting marked inhibition in nonhemolytic doses and no inhibition in hemolytic doses.

Substances toxic for rabbits, rats or mice could not be demonstrated in the blood of patients suffering with lobar pneumonia.

Neither pneumococcus protein nor pneumonic blood was found to have any neutralizing action on the toxicity of quinin salts for animals.

We regard as a tenable hypothesis, the view that the pneumotoxin may have the specific role of increasing the permeability of the walls of the capillaries lining the alveoli, for the various normal blood proteins, erythrocytes, leukocytes, fibrin and enzymes which go to make up the pneumonic lung exudate; and that it furthermore tends to inhibit the autolysis initiated by the proliferatig pneumococci, thus producing toxic albumoses, proteoses and peptones. At the crisis this inhibitory action is changed to an accelerating one and resolution then commonly follows.

Neither the clinically beneficial action of cinchona derivatives in the pneumonias nor the increased quinin tolerance of pneumonia patients receives elucidation from the experiments reported; the results being largely negative. Certain suggestive phenomena were observed that further study may elucidate. That cinchona derivatives are specifically pneumococcidal and that they increase phagocytic activity has been shown in previous papers. Whether their influence on general metabolism and on general antibody production will suffice to explain their further protective and curative influence remains to be determined.

THE CHEMOTHERAPY OF EXPERIMENTAL PNEUMO-COCCUS INFECTION

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The investigations of Morgenroth and his associates on the treatment of experimental pneumococcus infections with various derivatives of quinin and particularly ethylhydrocuprein, coupled with the researches of recent years on the differentiation of pneumococci by serologic methods and the serum treatment of lobar pneumonia, have renewed interest in the possibilities of discovering an efficient chemotherapeutic agent for this infection. A review of the literature on the treatment of pneumonia shows that a large variety of different drugs have been advocated from time to time in treatment on empiric grounds, while very few have been subjected to vigorous laboratory tests with an acceptable technic, for their possible influences on experimental pneumococcus infections. While it is obvious that the treatment of lobar pneumonia presents certain features widely divergent from the pneumococcus bacteriemia of experimental animals, and that certain drugs may exert a favorable influence in the general treatment of pneumonia which are without demonstrable pneumococcidal properties in vitro or in vivo, yet the efforts of the experimental chemotherapeutist must be in the direction of producing drugs possessing high pneumococcidal activity and of sufficiently low toxicity to permit the administration of doses capable of increasing the pneumococcidal activity of the body fluids in the living animals to such degree as will exert definite protective and curative effects against virulent pneumococci in the blood and tissues.

At the present time only derivatives of quinin are known to meet these requirements to any extent worthy of consideration. Advocated in the treatment of pneumonia about 40 years ago by von Jürgensen, Schultz, Aufrecht and Petzold and others, quinin compounds have continued in the favor of a number of practitioners in the treatment of lobar pneumonia, and within recent years Gibson, Galbraith and particularly S. Solis-Cohen, have been warm advocates of these com-

pounds as holding a place of definite value in the treatment of this infection.

As the investigations of Morgenroth and Levy[1] resulting in the discovery of ethylhydrocuprein (optochin), a synthetic derivative of hydroquinin (methylhydrocuprein) which latter exists in the cinchona bark or can be prepared synthetically, have definitely shown the high pneumococcidal properties of this substance in vitro and in vivo, results which have been generally corroborated by the investigations of Guttmann,[2] Morgenroth and Kauffmann,[3] Levy,[4] Wright,[5] Moore,[6] Cohen, Kolmer and Heist,[7] and others, it would appear that a derivative of quinin superior to ethylhydrocuprein may be discovered, which will exert sufficiently powerful and specific effects as to prove of definite value in the treatment of pneumonia in nontoxic doses.

Attracted by the high germicidal activity in vitro of compounds of mercury for various micro-organisms, calomel and bichlorid of mercury have been advocated empirically by Edelheit, Lepine and others in the treatment of lobar pneumonia, the latter injecting 1 c c of a 1 : 4,000 solution of sublimate directly into the involved lung. These mercurial compounds have not, however, proven of value in the treatment of lobar pneumonia.

EXPERIMENTS WITH NEW MERCURIAL COMPOUNDS

During the past year we have investigated the possible pneumococcidal properties of various new compounds of mercury prepared by Drs. Jay F. Schamberg, George W. Raiziss and John A. Kolmer in the Dermatological Research Laboratories of the Philadelphia Polyclinic, during the course of systematic researches on the chemotherapy of mercurial compounds in experimental trypanosomiasis.[8] We have found that these compounds possess well marked bactericidal properties for pneumococci in the test tube comparable to their bactericidal activity in vitro for staphylococci and typhoid bacilli, as reported by Schamberg, Kolmer and Raiziss.[8] The new compounds of mercury which we have tested for possible protective and curative properties

[1] Berl. klin. Wchnschr., 1911, 48, p. 1560 and 1970.
[2] Ztschr. f. Immunitätsf., O., 1912, 15, p. 625.
[3] Centralbl. f. Bakteriol., I, Ref., 1912, 54, p. 69.
[4] Berl. klin. Wchnschr., 1912, 49, p. 2486.
[5] On the Pharmacotherapy and Preventive Inoculation Applied to Pneumonia in the African Native, 1915.
[6] Jour. Exper. Med., 1915, 22, p. 269 and 551.
[7] Jour. Infect. Dis., 1917, 20, p. 272 and 313.
[8] Amer. Jour. of Syphilis, 1917, 1, p. 1.

against experimental pneumococcus infections in mice in addition to
the ordinary salts of mercury, have included combinations of mercury
with naphthol and naphthylanine, phenol, benzidine, aminophenyl-
arsenic acid, and guaiacol.

The toxicity of these compounds having been determined, our plan
of procedure was to administer graded doses to white mice according
to body weight by subcutaneous or intramuscular injection, two hours
before the intraperitoneal injection of a lethal dose of pneumococci.
We are indebted to Dr. Raiziss for his cooperation in preparing the
solutions of these new compounds for administration. In every experi-
ment numerous controls were included.

Throughout our investigation a Type I strain of pneumococcus
was employed; before each series of experiments the M. L. D. of a
24-hour broth culture was determined and the strain maintained by
animal passage at such virulence that 0.000,001 c c killed within 72
hours.

Attempts to use a smaller dose of culture in order to prolong the
lives of the controls by reason of a less severe infection, were generally
unsuccessful inasmuch as mice used as controls not infrequently
recovered spontaneously and therefore rendered very difficult a correct
interpretation of the effects of the compounds under study.

Without detailing our experiments or presenting tables showing
the results, we may state that all of the ordinary soluble salts of
mercury and a large number of the new compounds were found by
us to be without appreciable effect in prolonging the lives of mice
against pneumococcus bacteremia of sufficient severity to kill the con-
trols within 72 hours after infection. Not infrequently the larger
doses of these compounds appeared to hasten the deaths of the mice
by reason of the summation of the toxic effects of the drug and viru-
lence of the pneumococci; of the new compounds tested, the com-
bination of mercury and guaiacol appeared in some experiments to
prolong the lives of mice, but, in general, the results were of a negative
character.

EXPERIMENTS IN THE CHEMOSEROTHERAPY OF EXPERIMENTAL PNEUMOCOCCUS INFECTIONS

Investigations in the chemotherapy of pneumococcus infections
have indicated that only cinchona compounds or derivatives have any
promise as bases and leads in the development of an efficient chemo-
therapy for this infection. At the present time a derivative of hydro-

quinin, methylhydrocuprein (Morgenroth's optochin), which occurs in cinchona bark or may be prepared synthetically, and its soluble salt ethylhydrocuprein hydrochlorid, have proven most pneumococcidal in the test tube and in the living animal, although the high toxicity of these drugs has not permitted the administration of sufficient amounts to consistently influence lobar pneumonia. Furthermore, as found by Cohen, Kolmer and Heist,[7] the common compounds of quinin, as hydroquinin, and quinin and urea hydrochlorid, possess a high pneumococcidal activity in vitro and a slight degree of protective power in experimental pneumococcus infections.[9]

Observing that antipneumococcus serum for Type II pneumococci was less efficient than the serum for Type I, Moore[10] studied the influence of a combined optochin and serum treatment for Type II infections among mice, and found that a single dose of optochin suspended in oil and injected subcutaneously, which by itself has practically no protective effect, is capable of increasing the threshold value of Type II serum at least 50 times, and that this effect was proportionately many times greater than a simple summation of the protective and curative effects of the two substances separately. Previous work by Neufeld and Engwer,[11] Engwer[12] and Boehucke[13] also indicated a marked increase of protective and curative effects in mice by combining serum and drug therapy, although it is not clear from their publications that they used homologous strains of pneumococci and antiserums, and, as shown by Moore, an increased effect from the combined drug and serum treatment (chemoserotherapy) cannot be demonstrated unless the antiserum is homologous with the strain of pneumococci producing the infection.

In our studies we have used a virulent Type I strain of pneumococcus and the homologous antiserum combined with ethylhydrocuprein hydrochlorid and several of the commoner compounds of quinin, as the hydrochlorid, quinin and urea hydrochlorid, and hydroquinin; also combinations of the serum with several of the new mercurial compounds prepared in the Dermatological Research Laboratories of the Philadelphia Polyclinic by Schamberg, Raiziss and Kolmer.[8] While a number of these compounds were found without appreciable effect

[9] Jour. Infect. Dis., 1917, 20, p. 313.
[10] Jour. Exper. Med., 1915, 22, p. 389.
[11] Berl. klin. Wchnschr., 1912, 49, p. 2381.
[12] Ztschr. f. Hyg. u. Infektionskr., 1913, 73, p. 194.
[13] München. med. Wchnschr., 1913, 60, p. 398.

against virulent pneumococcus infections among mice,[8] their pneumococcidal values *in vitro* are very high, and we have considered it worth while to determine whether small doses would appreciably increase the protective and curative value of Type I serum.

Each of our experiments were conducted by first determining the minimal lethal dose of 24-hour broth culture of a Type I pneumococcus in 4 days for mice or rats or both, and then the protective power of an antipneumococcus serum in constant dose of 0.05, 0.1 or 0.2 c c against multiple doses of culture during the same interval of time. The animals were then given an intraperitoneal injection of a mixture of culture and antiserum followed immediately with a subcutaneous injection of the drug under study; numerous controls received culture alone; culture and antiserum without drug, culture and drug without antiserum, and drug without culture and antiserum. The results were judged according to the duration of life and the results of bacteriologic examination of the blood of the heart of those animals succumbing during the period of the experiment. As shown by Table 1, we found that 0.1 c c of antiserum (Rockefeller Institute)

TABLE 1

The Effect of Ethylhydrocuprein Hydrochlorid by Subcutaneous Injection on the Protective Value of Antipneumococcus Serum for Type I Pneumococci

Weight in Gm.	Culture in C C	Serum in C C	Drug per 100 Gm. of Body Weight	Results
17	0.05	0.1	—	Died in 48 hours
16	0.1	0.1	—	Died in 48 hours
21	0.2	0.1	—	Died in 24 hours
19	0.1	0.1	0.002	Survived
23	0.2	0.1	0.002	Died in 96 hours
20	0.05	None	0.002	Died in 24 hours

injected intraperitoneally (equivalent to about 300 c c per 60 kg.) protected mice against 0.1 c c of culture, about 100,000 minimum lethal doses, for 24 hours only, whereas with the same amount of antiserum the subcutaneous injection of 0.002 gm. ethylhydrocuprein hydrochlorid per 100 gm. of mouse (equivalent to 1.2 gm. per 60 kg.) protected for at least 4 days against this dose of culture and for 3 days against 0.2 c c of culture, equal to 200,000 M. D. Other experiments yielded similar results and demonstrated that amounts of ethylhydrocuprein hydrochlorid too small to afford protection against large doses of virulent pneumococi, apreciably increase the protective value of homologous antiserum.

TABLE 2

The Effect of Quinin and Urea Hydrochlorid by Subcutaneous Injection on the Protective Value of Antipneumococcus Serum for Type I Pneumococci

Weight in Gm.	Culture in C C	Serum in C C	Drug per 100 Gm. Body Weight	Results	Heart Blood
77	0.5	0.05	—	Died in 24 hours	
48	0.4	0.05	—	Died in 24 hours	Pneumococci
82	0.3	0.05	—	Died in 96 hours	Pneumococci
46	0.2	0.05	—	Survived	
83	0.5	0.05	0.005	Died in 48 hours	Pneumococci
70	0.4	0.05	0.005	Died in 48 hours	Pneumococci
80	0.3	0.05	0.005	Died in 72 hours	
95	0.2	0.05	0.005	Survived	
60	0.4	0.05	0.0025	Died in 24 hours	Pneumococci
119	0.3	0.05	0.0025	Survived	Pneumococci
90	0.2	None	0.005	Died in 24 hours	

We found further that 0.05 c c antiserum intraperitoneally, equivalent to 150 c c per 60 kg., protected a white rat against 0.3 c c culture, 3,000 M.D.D., for 3 days and against 0.2 c c of culture, 2,000 M.D.D., for at least 6 days (Table 2); the administration of quinin and urea hydrochlorid in dose of 0.005 gm. per 100 gm. of rat, equivalent to 3 gm. per 60 kg., appeared to exert a slight influence against the higher doses of culture and 0.0025 gm. per 100 gm. of body weight, equivalent to 1.5 gm. per 60 kg., not infrequently prolonged the lives of animals against smaller doses of culture beyond the protection afforded by serum alone. These results are typical of those observed with the other quinin compounds tested, that is, the influence they exerted was usually irregular and feeble.

In the experiment with mice (Table 3) 0.0001 c c of culture alone was fatal within 72 hours; 0.5 c c of antiserum protected against 2,000 fatal doses of culture (0.2 c c) for at least 5 days while the same amount of serum protected against 4,000 doses of culture (0.4 c c) for 2 days and yielded no protection for 5,000 fatal doses of culture (0.5 c c). A second series of mice receiving similar decreasing doses of culture mixed with a constant dose (0.05 c c) of serum followed by the subcutaneous injection of 0.001 gm. of etylhydrocuprein hydrochlorid per 100 gm. of mouse (equivalent to 0.6 gm. per 60 kg.) showed a distinct tendency toward prolonging the lives of mice, while similar doses of quinin and urea hydrochlorid and quinin chlorohydrosulphate also appeared to prolong the lives of several animals although the results were too irregular in all experiments to enable us to express them in more definite terms. All of these drugs alone in the same dosage, 0.001 gm. per 100 gm. of mouse, and injected subcutaneously had no appreciable effect on the large doses of culture employed.

TABLE 3

THE INFLUENCE OF ETHYLHYDROCUPREIN HYDROCHLORID, QUININ AND UREA HYDROCHLORID, AND QUININ CHLOROHYDROSULPHATE BY A SUBCUTANEOUS INJECTION OF 0.001 GM. OF BODY WEIGHT ON THE PROTECTIVE VALUE OF ANTIPNEUMOCOCCUS SERUM AGAINST INCREASING DOSES OF PNEUMOCOCCI TYPE I IN WHITE MICE

Culture in C C	Serum in C C	Ethylhydro-cuprein Hydrochlorid	Quinin and Urea Hydrochlorid	Quinin and Chlorohydro-sulphate	Result
0.1	—	—	—	—	Died in 24 hours
0.01	—	—	—	—	Died in 24 hours
0.001	— —	—	—	—	Died in 48 hours
0.0001	—	—	—	—	Died in 72 hours
0.5	0.5	—	—	—	Died in 24 hours
0.4	0.5	—	—	—	Died in 72 hours
0.3	0.5	—	—	—	Died in 120 hours
0.2	0.5	—	—	—	Survived 120 hrs.
0.5	0.5	+	—	—	Died in 72 hours
0.4	0.5	+	—	—	Survived 120 hrs.
0.3	0.5	+	—	—	Died in 96 hours
0.2	0.5	+	—	—	Survived 120 hrs.
0.5	0.5	—	+	—	Died in 72 hours
0.4	0.5	—	+	—	Died in 72 hours
0.3	0.5	—	+	—	Died in 120 hours
0.2	0.5	—	+	—	Died in 120 hours
0.5	0.5	—	—	+	Died in 48 hours
0.4	0.5	—	—	+	Survived 120 hrs.
0.3	0.5	—	—	+	Died in 72 hours
0.2	0.5	—	—	+	Survived 120 hrs.

TABLE 4

THE INFLUENCE OF ETHYLHYDROCUPREIN HYDROCHLORID, QUININ AND UREA HYDROCHLORID, AND QUININ HYDROBROMID BY SUBCUTANEOUS INJECTION ON THE PROTECTIVE VALUE OF ANTIPNEUMOCOCCUS SERUM AGAINST A CONSTANT LARGE DOSE OF PNEUMOCOCCUS OF TYPE I IN WHITE RATS

Culture in C C	Serum in C C	Cinchona Compound 0.004 Gm. for Each 100 Gm. of Weight	Result
0.1	0.2	—	Died in 48 hours
0.01	0.2	—	Died in 48 hours
0.001	0.2	—	Died in 72 hours
0.0001	0.2	—	Survived 96 hours
0.1	0.2	Ethylhydrocuprein	Died in 96 hours
0.01	0.2	Hydrochlorid	Died in 48 hours
0.001	0.2	....	Died in 24 hours
0.0001	0.2	....	Survived 96 hours
0.1	0.2	....	Died in 48 hours
0.01	0.2	Quinin and	Died in 48 hours
0.001	0.2	Urea Hydrochlorid	Died in 24 hours
0.0001	0.2	....	Survived 96 hours
0.1	0.2		Died in 48 hours
0.01	0.2	Quinin	Survived 96 hours
0.001	0.2	Hydrobromid	Died in 24 hours
0.0001	0.2	....	Survived 96 hours

The results of experiments (Table 4) with white rats weighing from 80-130 gm., indicated that 0.2 c c of a serum for Type I pneumococcus protected against 0.1 c c of culture for 24 hours and against 0.0001 c c of culture for more than 96 hours; this strain was of such virulence that 0.0001 c c per 100 gm. of rat killed in 48 hours. Both culture and serum were injected intraperitoneally. Intraperitoneal injection of 0.1 c c of culture per 100 gm. of rat, equivalent to 1,000 M.L.D., with 0.2 c c of serum per 100 gm., equivalent to 120 c c per 60 kg., followed immediately by the subcutaneous injection of 0.004

TABLE 5

The Influence of Mercurial Compounds Administered by Subcutaneous Injection on the Protective Value of Antipneumococcus Serum for Type I Pneumococci in White Rats

Culture in C C	Serum in C C	New Mercurial Compound and Dose in Gm. for Each 100 Gm. of Weight	Results
0.1	—	—	Died in 24 hours
0.01	—	—	Died in 24 hours
0.001	—	—	
0.3	0.5	—	Survived 120 hours
0.3	0.5	—	Survived 120 hours
0.3	0.5	—	Died after 120 hours
0.3	0.5	Compound 99—0.01	Died in 72 hours
0.3	0.5	Compound 99—0.0005	Died in 96 hours
0.3	0.5	Compound 99—0.00025	Survived 120 hours
0.3	0.5	Compound 105—0.005	Died in 120 hours
0.3	0.5	Compound 105—0.0005	Died in 96 hours
0.3	0.5	Compound 105—0.00025	Died in 96 hours
0.3	0.5	Compound 42—0.001	Died in 24 hours
0.3	0.5	Compound 42—0.0005	Survived 120 hours
0.3	0.5	Compound 42—0.00025	Died in 96 hours
0.3	0.5	Compound 112—0.001	Survived 120 hours
0.3	0.5	Compound 112—0.0005	Died in 120 hours
0.3	0.5	Compound 112—0.00025	Died in 96 hours
0.3	0.5	Bichl. mercury—0.001	Died in 120 hours
0.3	0.5	Bichl. mercury—0.0005	Died in 96 hours
0.3	0.5	Bichl. mercury—0.00025	Died in 120 hours

gm. of ethylhydrocuprein hydrochlorid per 100 gm. of rat, equivalent to 2.4 gm. per 60 kg., protected a rat for 72 hours and another for 24 hours; the serum alone protected for 24 hours but never for a longer period. Quinin and urea hydrochlorid in equal dose in similar experiments exerted no appreciable effects, while quinin hydrobromid in the same amount protected a rat for a period beyond 96 hours. The drugs alone were not toxic and afforded no protection against this large dose of culture.

The results of one experiment (Table 5) of injecting serum and culture intraperitoneally and graded doses of various known and new

preparations of mercury which possess high pneumococcidal powers in vitro subcutaneously, may be given as follows: The culture of Type I pneumococcus employed killed rats weighing from 80-130 gm. in dose of 0.001 c c in 72 hours; 0.05 c c of serum protected against 0.3 c c of culture, 300 M.L.D., for at least 4 days or longer. Bichlorid of mercury administered subcutaneously immediately after the intraperitoneal injection of a mixture of 0.3 c c culture and 0.05 c c serum, did not prolong the lives of any mice; occasionally rats receiving subcutaneous injections of graded doses of several of the new synthetic mercurial compounds lived indefinitely, but the results were always similar to those shown in this table and so irregular and of such a nature as to permit of nothing more than the tentative deduction that several of these compounds in small doses appeared to slightly increase the protective effect of the serum; as previously reported[8] these compounds alone had no appreciable effect on pneumococcus injections among rats and mice.

SUMMARY

The ordinary soluble salts of mercury and numerous new mercurial compounds were found to be without appreciable effect in prolonging the lives of mice infected with a dose of Type I pneumococcus culture lethal within 72 hours.

While systematic researches in the chemotherapy of pneumococcus infections have not been numerous and a large number of different substances cannot be said to have been tried out experimentally, the results of investigations to the present time indicate that derivatives of quinin constitute the best bases or leads in the development of the chemotherapy of pneumococcus infections. It appears that systematic investigations with these compounds is full of promise in the field of chemotherapeutic research of bacterial infections in general and of pneumococcus infections in particular.

The results of our experiments indicate that ethylhydrocuprein hydrochlorid by subcutaneous injection in doses without protective value, usually increase the protective value of antipneumococcus serum Type I in a slight but definite manner in severe infections of mice and rats with homologous pneumococci.

Several of the commoner compounds of quinin, as quinin and urea hydrochlorid, quinin bromid, and quinin chlorohydrosulphate, given subcutaneously in doses without any appreciable influence on severe

and fatal pneumococcus infections, occasionally increase the protective power of antipneumococcus serum but to a lesser extent and less regularly than ethylhydrocuprein hydrochlorid.

Ordinary salts of mercury subcutaneously did not increase the protective value of antipneumococcus serum; several new synthetic compounds of mercury were generally without effect on the protective power of the serum in severe experimental infections.

LEUKOPENIA AND LEUKOCYTOSIS IN SPLENEC-
TOMIZED RABBITS

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In a previous report[1] it was shown that shortly after the intra-
venous injection of killed bacteria in rabbits there results a marked
decrease in the leukocytes involving chiefly the polymorphonuclear
elements, and that simultaneously the blood from the spleen and liver
contain an enormously increased number of leukocytes, especially the
polymorphonuclears. In view of this observation it seemed of interest
to make a similar study on splenectomized rabbits.

As a rule the blood of normal rabbits from other sources than the
splenic parenchyma and the veins of the principal organs contains a
fairly uniform number of leukocytes; the blood from the organs
except the spleen containing slightly more than that from the spleen
and several times the number in the general blood stream.

Eight adult rabbits were used. Leukopenia was induced by the
intravenous injection of 40-60 million of dead typhoid bacilli or strep-
tococci, the injections being made from 1-94 days after splenectomy
(Table 1). Leukopenia would develop from 3-10 minutes after the
injection. In Rabbit 2 a drop from 15,150 to 1,300 leukocytes in
each c.mm. of the peripheral blood occurred within 4 minutes. During
the leukopenia the number of leukocytes in the blood in the bone
marrow were 2-5 times as many, in the superior mesenteric vein and
artery and in the liver parenchyma twice as many, in the jugular vein
4 times as many, and in the hepatic and portal vein twice as many as
in the peripheral blood; the number in the blood of the muscles, the
kidney parenchyma, the left and right ventricles, the lung parenchyma,
the hepatic artery, the superior and inferior vena cava, and the axil-
lary artery corresponded to that in the ear vein.

In Rabbits 2, 5, 7, and 9 there occurred a decrease in the number
and proportion of the polymorphonuclear leukocytes in the blood from
all sources, including that from the bone marrow, which was not the

case in the nonsplenectomized rabbits. In Rabbits 1, 8, and 10 there occurred a decrease in the number and proportion of the polymorphonuclear leukocytes in the blood from all sources examined except from the bone-marrow, the liver and the lung parenchyma. In the case of the liver and lung parenchyma the proportion of the polymorphonuclear elements dropped synchronously with the drop in the blood from other sources; but in the case of the bone-marrow the proportion of these elements remained normal. In Rabbit 6, the injection being made the day following splenectomy, there occurred only a slight drop in the number and proportion of the polymorphonuclear leukocytes in the blood from all sources examined, suggesting an unbalanced condition of the hemopoietic system on account of the splenectomy. In the other rabbits the splenectomy had been made 7-94 days before the injection.

TABLE 1

Leucocyte Counts in Splenectomized Rabbits During Leukopenia Following Intravenous Injection of Bacteria

	Rabbit 8	Rabbit 9	Rabbit 10	Rabbit 7	Rabbit 6	Rabbit 5	Rabbit 2	Rabbit 1
Bacterial injection..........	60 million living streptococci	40 million dead streptococci	60 million dead typhoid bacilli	60 million dead typhoid bacilli	60 million dead typhoid bacilli	50 million dead typhoid bacilli	60 million dead typhoid bacilli	60 million dead typhoid bacilli
Days since splenectomy.....	57	32	8	7	1	14	9	94
Leukocytes in blood of ear vein before injection......	12,500	9750	10,300	9350	13,000	10,850	15,150	11,500
Leukocyte counts during leukopenia:								
Ear vein...............	.....	5000	1750	2250	2000	4150	1300	3300
Bone marrow..........	44,200	7700	8400	4850	6850	7650	2850	6050
Muscle.................	.....	.....	.....	.....	.....	.....	.....	3100
Sup. mesent. vein.....	.....	.....	.....	5250	4050	6650	3000	4000
Sup. mesent. artery...	.....	.....	.....	4750	2200	6100	2600	2500
Liver..................	20,300	9500	5100	5450	2200	10,400	5000	12,150
Kidney.................	.....	.....	2600	.....	.....	.....	.....	4450
Left ventricle..........	.....	.....	1200	5000	3150	2400	1150	2200
Right ventricle........	.....	.....	1500	4350	2600	2800	3100	1300
Lung..................	11,650	4750	1450	5700	4550	5800	1600	3500
Jugular vein...........	.....	.....	4350	.....	.....	.....	.....	13,900
Hepatic vein...........	.....	.....	.....	.....	.....	.....	4500	.....
Hepatic artery........	.....	.....	.....	3400	.....	4950	.....	.....
Sup. vena cava........	.....	.....	.....	8300	3550	4100	4550	.....
Inf. vena cava........	.....	.....	.....	3450	3700	4700	.....	.....
Portal vein............	.....	.....	.....	5500	.....	.....	.....	.....
Left axil. vein.........	.....	5450	.....	.....	.....	.....	.....	.....

In all the rabbits the proportion of small mononuclear leukocytes was greatly increased, but the actual number remained practically normal, except in Rabbit 6 (injected one day after the splenectomy); in this case there occurred a decrease. No material change was noted as to the large mononuclear leukocytes.

TABLE 2

DIFFERENTIAL COUNTS OF LEUKOCYTES DURING LEUKOPENIA IN SPLENECTOMIZED RABBIT
(TABLE 1, RABBIT 2)

Source and Time	Leuko-cytes per C Mm	Differential Counts								Remarks
		Poly-morpho-nuclear		Small Lympho-cytes		Large Lympho-cytes		Mast Cells		
		No.	%	No.	%	No.	%	No.	%	
Left ear vein 1 day before splenectomy	12,000	3538	29	6588	54	1708	14	244	2	
Left ear vein 1 day after splenectomy	15,150	4242	28	6666	44	2121	26	303	2	
Left ear vein 9 days after splenectomy	5950	2737	46	1904	32	1071	18	238	4	
Left ear vein 5 minutes after injection of 60 million killed typhoid bacilli..	1300	0	0	1144	88	143	11	13	1	
Bone-marrow	2850	200	7	2280	80	199	7	171	6	
Sup. mes. vein	3000	30	1	2310	77	420	14	240	8	
Sup. mes. artery	2600	0	0	1716	66	520	20	364	14	13 to 23 minutes after injec-
Liver	5000	150	3	4000	80	250	5	600	12	
Lung	1600	32	2	1280	80	144	9	144	9	
Hepatic vein	4550	228	5	3822	84	364	8	136	3	
Left ventricle	1150	69	6	920	80	23	2	138	12	
Right ventricle	3100	31	1	2294	74	527	17	248	8	
Sup. vena cava	4550	136	3	3913	86	409	9	91	2	

In several instances the lungs examined were normal; a differential count of the leukocytes in cross-section of an artery showed 104 polymorphonuclear leukocytes and 218 mononuclear leukocytes; the count in a large vein was polymorphonuclear leukocytes 2, mononuclear leukocytes 45. In some instances marked congestion of the capillaries was present without noticeable increase of leukocytes or edema.

No increase in the number of leukocytes was noted in the liver. Bacteria were not seen. The large blood vessels contained largely lymphocytes, there being a slight preponderance of the polymorphonuclear leukocytes in the small vessels. A differential count of the leukocytes in a medium sized artery showed 45 polymorphonuclear and 50 mononuclear cells.

The bone-marrow occasionally showed moderate hyperemia and hyperplasia; the giant cells appeared normal.

Kidneys, lymph nodes, parotid and thymus glands appeared normal.

The pharyngeal mucosa showed marked infiltration of the submucosa and subjacent areolar tissue with polymorphonuclear leukocytes.

During the leukocytosis in splenectomized rabbits from intravenous injections of dead bacteria there occurred no appreciable variation in the relation of the various elements from that during the same stage in

nonsplenectomized rabbits. There occurs a marked general increase in the total number of leukocytes, as well as in the number and proportion of the polymorphonuclear cells, the increase being least in the blood from the left ventricle. The number of small and large lymphocytes was not appreciably increased in the blood from any of the sources studied, but the blood from both sides of the heart contained fewer of these elements than the blood from the other sources. The mast cells were generally slightly increased over the normal.

TABLE 3

Leukocyte Counts During Hyperleukocytosis in Splenectomized Rabbits

Source and Time	Total Leuko-cytes per C Mm	Differential Counts							
		Polymorpho-nuclears		Small Mononuclears		Large Mononuclears		Mast Cells	
		No.	%	No.	%	No.	%	No.	%
Ear vein 24 hours before splenectomy.............	9750	1755	18	5168	53	1170	12	1657	17
Ear vein 24 hours after splenectomy.............	8900	3847	43	2225	25	1602	18	1246	14
Ear vein before intra-venous injection of 40 million dead typhoid bacilli..........	10,750	6128	57	3117	29	1290	12	215	2
24 hours after injection of typhoid bacilli:									
Left ear.............	45,300	38,505	85	3171	7	3171	7	453	1
Bone marrow........	39,500	28,835	73	5135	13	4740	12	790	2
Sup. mes. vein.......	32,000	25,600	80	2560	8	3520	11	320	1
Sup. mes. artery....	35,500	29,820	84	3905	11	1775	5	0	0
Liver................	32,200	24,150	75	4508	14	3542	11	0	0
Lung................	29,700	23,166	78	3564	12	2376	8	594	2
Left ventricle........	19,900	15,711	79	1990	10	1791	9	398	2
Right ventricle......	24,200	20,086	83	1936	8	1694	7	484	2
Sup. vena cava......	29,800	21,158	71	5364	18	2384	8	894	3
Inf. vena cava......	22,400	17,472	78	2688	12	2240	10	0	0

CONCLUSIONS AND SUMMARY

The principal result from this study would seem to be the observation that there is no variation in the reaction of the leukocytes in splenectomized rabbits from that in nonsplenectomized rabbits, after intravenous injections of dead bacteria. In both instances a leukopenia occurs within a few minutes following the injection, followed a few hours later by a marked leukocytosis. The length of time elapsing between the splenectomy and the injection apparently has no bearing on the severity of the reaction. There seems to be a tendency to a slight increase in the total number of leukocytes following splenectomy.

The leukocytes in the blood from the bone-marrow and liver parenchyma during the leukopenia were decreased somewhat, except

in one case (Rabbit 8), in which there was a marked increase in the total number and also in the proportion and number of polymorphonuclear cells. This rabbit, however, received an injection of living Streptococcus viridans, while the other rabbits received injections of dead hemolytic streptococci and typhoid bacilli.

The presence of large numbers of polymorphonuclear cells in the sections of the peripharyngeal tissues during leukopenia may be explained as due to the local irritating action of the ether employed for anesthesia.

No satisfactory explanation is apparent as a result of this study for the fate of the polymorphonuclear cells during the leukopenia occurring in splenectomized rabbits following intravenous injections of bacteria.

ACID PRODUCTION AT PARTIAL OXYGEN TENSION AND UNDER AEROBIC CONDITIONS BY A BACILLUS OF THE TYPHOID DYSENTERY GROUP

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This article deals with the fermentative reactions of a bacillus from the feces of a case of dysentery. Morphologically and culturally, the bacillus shows the characteristics of the dysentery bacillus. It is a short, nonmotile, gram-negative rod, with a typhoidlike growth on plain agar and gelatin, producing a clouding of broth with a small amount of deposit at the end of 3-5 days' incubation at 37 C. Milk is not coagulated and after a short initial acidity, the milk becomes more alkaline, as shown by a deepening of the blue color of the litmus. Gelatin is not liquefied and no gas is produced in carbohydrate mediums.

Four days after isolation, the fermentative characters of the organism were tested in sugar broths ($+$ 1 meat extract broth, previously rendered sugar free by inoculation with B. coli, to which were added the respective sugars, in approximately 1% amounts, and sufficient litmus to impart a definite blue color to the solution). At the end of 48 hours' incubation at 37 C. acid production was marked in dextrose. In mannite and saccharose there was a trace of acid, with partial reduction of litmus. After 4 days' incubation at 37 C. the dextrose broth was still markedly acid, but the reaction in mannite and saccharose had become alkaline. At the end of 9 days' incubation the dextrose tube alone showed acid production. Lactose, maltose, and dextrin remained alkaline. A second series of sugar broths inoculated from a plain agar aerobic slant, which was the second transplant on plain agar after the organism was obtained in pure culture, showed acid production in dextrose and alkali in lactose, maltose, mannite, saccharose, and dextrin, after 48 hours' incubation. After three transfers on aerobic plain agar ($+$ 1) slants, the organism was grown on plain agar ($+$ 1) at partial oxygen tension by connecting it and incubating it with a slant inoculated with B. subtilis.[1] The 48-hour growth at partial tension was marked but not quite so pronounced as on plain agar incubated aerobically. After continuous incubation on plain agar at partial tension for 2 days, transplants were made to slants of litmus sugar-ascitic Martin agar.[2] The medium was prepared as follows: sodium phosphate agar, 0.5% acid to phenolphthalein as recommended by W. Blair M. Martin, was rendered sugar

[1] Wherry and Oliver: Jour. Infect. Dis., 1916, 19, p. 288, and 1917, 20, p. 28.
[2] W. Blair M. Martin: Jour. Path. and Bacteriol., 1911, 15, p. 76.

free by inoculation with B. coli. After filtering and heating, ascitic fluid was added in the proportion of about one of fluid to four of the agar, then the sugars, to a concentration of about 1%, and then sufficient litmus to color the medium. The tubes were slanted.

On 24 hour incubation at partial oxygen tension, acid production in dextrose was marked, but lactose, galactose, maltose, mannite, levulose, and saccharose remained alkaline. On further incubation at partial tension for 48 hours not only did the dextrose slant show marked acid production, but likewise in the other sugars marked acid reaction had appeared, which persisted.

These findings were so unexpected that the partial tension sugar agars were examined microscopically with Gram's stain and were also plated out in plain agar to test the suspicion that we were working with a mixed culture. The plates showed a pure culture of a gram-negative rod which corresponded morphologically and culturally with the organism described as isolated from the stool.

After 3 days' incubation at 37 C. transplants were made to a second series of litmus sugar-ascitic Martin agar slants which were made at the same time and in a manner identical with those of the first series. Transplants to the sugar agars were made from the respective sugar agars of Series 1, namely, from dextrose to dextrose, mannite to mannite, etc. This second series (Series 2) was made partial tension in the same manner as the first and was then incubated at 37 C. Transplants (Series 3) were also made from partial tension sugars of the first series to respective sugar litmus broths ($+1$) (Series 3) which were incubated aerobically at 37 C.

Forty-eight hours later, Series 2 (that is, the partial tension litmus sugar-ascitic Martin agar slants) showed marked acid production in dextrose, lactose, galactose, maltose, mannite, levulose, and saccharose. The aerobic sugar broth series (Series 3), on the other hand, showed acid production only in dextrose, thus corresponding with the original reactions obtained in aerobic sugar broths, when first tested after isolation from the stool. Thro advises against the use of ascitic fluid when testing the variability of micro-organisms on the carbohydrates, because he found that different samples of ascitic fluid vary in the amount of fermentable sugars that they contain. To overcome the objection that possibly the acid production obtained at partial tension in a variety of sugars might be due to the use of ascitic fluid in the medium,[3] transplants were made from the partial tension Series 2 to slants of sugar free agar ($+1$) to which respective sugars were added in a concentration of about 1%. Likewise, the same procedure was employed as regards the aerobic Series 3, in each case transfers being made from the respective sugars. Again, identical results to those described were obtained, that is, under aerobic conditions, dextrose alone was fermented, whereas under conditions of partial oxygen tension acid production occurred not only in dextrose, but in lactose, galactose, maltose, mannite, levulose, and saccharose.

DISCUSSION

One of the most intensive studies of the influence of environment on the characters of micro-organisms, especially of the colon group, is that by Peckham.[4] By modifying the environment she was able to exalt in some cases the activity of one function and in other cases to

[3] Thro, W. C.: Jour. Infect. Dis., 1915, 17, p. 227.
[4] Jour. Exper. Med., 1897, 2, p. 550.

depress or even extinguish certain biologic traits. By a series of cultural experiments, for instance, she was able to force B. typhosus to produce indol.

Twort,[5] in a study of bacterial variations, claimed that by prolonged cultivation on saccharose of different strains of the typhoid, the paratyphoid, and dysentery bacilli, they all acquired the property of fermenting saccharose. After repeated transfers on saccharose the dysentery bacillus was able to produce acid from this sugar in 24 hours. It required 2 years of constant growth on lactose for Twort to produce a lactose-fermenting variant of B. typhosus.

In the case of an organism studied by Klotz,[6] belonging to the colon group isolated from water, which did not ferment lactose or saccharose when first isolated, repeated transfers into lactose and saccharose broth, lead finally to the acquisition by the organism of the power of fermenting these sugars, with acid and gas production.

In regard to the experimental inhibition of fermentative activities, Herter,[7] for instance, has shown that the fermentative activities of B. coli are considerably inhibited by the presence of sodium benzoate in weak glucose broth, whereas other biologic activities of the bacillus are but slightly, if at all, impaired. Sodium benzoate (0.1%) in dextrose broth only slightly or moderately inhibits the growth of B. coli, but gas production may, however, be considerably inhibited. Likewise, Penfold[8] found that sodium acetate inhibits the fermentative activities of B. coli, B. enteritidis and B. paratyphi, probably by inhibition or destruction of the enzyme invertase, as evidenced by the fact that although gas formation in the sugar was diminished and finally totally disappeared, yet the bacillus was still capable of producing gas from the corresponding alcohols. Smirnow[9] reports that continuous growth of B. coli in 3% glucose broth lead, in the case of 3 of 7 strains studied by him, to a complete inhibition of both acid and gas production in the following sugars: glucose, lactose, maltose, saccharose, dextrin, and mannite. In 2 other strains, glucose varied the amount of acid and gas production, with an occasional complete inhibition in some of the sugars.

A consideration of the foregoing work reveals what may be termed induced variations in the biologic activities of bacteria, especially as

[5] Proc. Roy. Soc., B., 1907, 79, p. 329.
[6] Jour. Infect. Dis., 1906, Sup. 2, p. 35.
[7] Jour. Biol. Chem., 1909, 7, p. 59.
[8] Brit. Med. Jour., 1911, Sup. 2, p. 363.
[9] Jour. Bacteriol., 1916, 1, p. 385.

regards gas production and acid production. The results detailed in our communication possibly are capable of a different interpretation.

Wherry and Oliver[1] have published observations which tend to establish the generalization that many bacterial as well as animal endo-parasites become adapted to a tension of oxygen below the atmospheric. Within the body organisms, as a general rule, multiply and exhibit their characteristic activities under conditions of lowered oxygen tension. Corroborative evidence to this effect is supplied by our observations that the isolation and cultivation on laboratory mediums of such organisms as the meningococcus, gonococcus, certain strains of streptococci, etc., can best, and in some cases, only be effected by growing these organisms under conditions of partial oxygen tension. Admittedly, the culture mediums we employ in the laboratory are at best a poor substitute for the habitat that bacteria find in the body. However, the more closely we simulate body conditions in the test tube or flask, the more accurately, it would seem, will we be able to demonstrate in cultures the biologic activities that micro-organisms exercise within the body. We would suggest that possibly, in the case of the organism described in the present communication, the production of acid in dextrose, lactose, galactose, maltose, mannite, levulose, and saccharose, under conditions of partial oxygen tension, whereas under aerobic conditions, permanent acid production occurred only in glucose, took place because the organisms grown at diminished oxygen tension were supplied with conditions more closely simulating those encountered in their habitat in the body. Viewed in this light, we would be dealing not with an example of induced variation in a biologic character.

In conclusion, it may be pointed out that in the case of the organism studied in this paper, we seem to be dealing with certain enzymes which function only under an oxygen tension below the atmospheric.

SUMMARY

An organism, culturally of the typhoid-dysentery group, isolated from the feces in a case of dysentery, when grown under aerobic conditions, produced persistent acid from dextrose alone, whereas, when grown at diminished oxygen tension, acid was produced in dextrose, lactose, galactose, maltose, mannite, levulose, and saccharose.

DIFFERENTIATION OF THE PARATYPHOID-ENTERITIDIS GROUP, IV

THE BEHAVIOR OF B. PARATYPHOSUS A AND B. PARATYPHOSUS B IN MILK

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It is now fairly definitely established that freshly isolated paratyphoid A strains slowly produce alkali in milk, and that alkali formation can always be observed when the incubation period is sufficiently prolonged.[1] On the basis of these results it is manifestly incorrect to speak of "permanent acidity" in litmus milk as a distinguishing characteristic of the paratyphoid A type. On the other hand, there is substantial agreement that the paratyphoid B strains produce alkali in milk noticeably earlier than the A strains. Gradations and variations in both types occur, especially in strains that have been for some time under artificial cultivation, but in general the distinction is fairly sharp. If a large series of strains be compared in litmus milk between the 4th and 8th days, it will be very rare indeed that the B. paratyphosus A strains cannot be separated readily from B. paratyphosus B, B. suipestifer and B. enteritidis. In the course of examination of between 200 and 300 strains from many sources, I have found but one strain that could not be so distinguished. This was a paratyphoid B strain (No. 221),[2] typical in all other respects, which does not begin to show alkalinity until about the 10th day and so overlaps some of the more rapid alkali-producing A strains, as for example two or three kindly sent me by Dr. Krumwiede.

In order to determine whether the different behavior of the A and B types in milk is connected with the rate of multiplication of these organisms, I have plated out a series of cultures at different ages.

Erlenmeyer flasks of 100 c c capacity and containing 50 c c of fresh certified milk sterilized in the Arnold were inoculated respectively with a number of strains of definitely ascertained characters. Counts were

[1] Bradley: Jour. and Proc. Roy. Soc. N. S. Wales, 1912, 46, p. 74. Krumwiede, Pratt and Kohn: Jour. Med. Research, 1916, 35, p. 52. Hadley: Jour. Bacteriol., 1917, 2, p. 263. Jordan: Jour. Infect. Dis., 1917, 20, p. 457.

[2] Jordan, E. O.: Jour. Infect. Dis., 1917, 20, p. 457.

made on agar plates after the milk had been incubated at 37 C. Table 1 shows a representative series. The figures are averages of counts of duplicate plates.

TABLE 1

Counts Taken in a Representative Series of Strains. Milk at 37 C.

	No. of Strain[2]	Initial Number Per Cc	After 3 Days	After 7 Days
Para B strains...	12	33,000	730,000,000	560,000,000
	185	26,000	355,000,000	320,000,000
	210	156,000	590,000,000	570,000,000
Suipestifer strains	63	94,000	391,000,000	160,000,000
	167	4,000	470,000,000	318,000,000
	234	589,000	630,000,000	270,000,000
Para A strains...	4	34,000	30,000,000	108,000,000
	158	63,000	23,000,000	83,000,000
	188	12,000	37,000,000	120,000,000

In another series two sets of flasks inoculated with Para B and Para A strains gave the counts described in Table 2.

TABLE 2

Counts Made of the Strains in Two Sets of Flasks. Milk at 37 C.

	Initial Number	After 5 Days	After 15 Days
2 Para B strains (12, 185) 2 flasks each. Average 4 plates...	98,000	830,000,000	103,000,000
2 Para A strains (198, 212) 2 flasks each. Average 4 plates...	90,000	69,000,000	250,000,000

Even when very heavy initial inoculations are made, some difference is apparent, as shown in the series given in Table 3.

TABLE 3

Showing the Gradations when Heavy Initial Inoculations Were Used. Milk at 37 C.

	Initial Number	After 3 Days	After 10 Days
4 Para B strains (12, 179, 180, 222) 2 flasks each	14,700,000	942,000,000	432,000,000
3 B. suipestifer strains (63, 118, 167) 2 flasks each	14,900,000	593,000,000	262,000,000
3 B. enteritidis strains (52, 206, 228) 2 flasks each	26,300,000	566,000,000	308,000,000
3 Para A strains (158, 198, 212) 2 flasks each	11,500,000	270,000,000	418,000,000

The multiplication of the Para A strains in milk is generally not as rapid and the numbers do not reach as high a point as is the case with the other members of the group. In this way nearly all the

strains described in an earlier paper have been subjected to trial, always with the same result.

On the basis of a large number of counts, into the details of which it does not seem necessary to go, the statement seems warranted that within the first five days after inoculation of approximately equal numbers, the number of Para A bacilli in milk is less than one-half or one-third and often less than one-tenth of the number of Para B bacilli. Connected with this is the fact that the numbers of Para A bacilli generally show an increase between the 3rd and 7th or 3rd and 10th day counts while the B strains show a decrease.

One further interesting observation may be noted. I have already referred to the relatively slow production of alkali by Strain 221. This is connected with a relatively slow multiplication as shown in the series noted in Table 4.

TABLE 4

COMPARISON OF THE RATE OF MULTIPLICATION OF SEVERAL STRAINS

	Initial Number	After 3 Days	After 7 Days
3 Para B strains (12, 185, 210)	72,000	558,000,000	466,000,000
Strain 221	441,000	210,000,000	160,000,000
3 Para A strains (9, 158, 219)......	38,000	31,000,000	76,000,000

TABLE 5

SHOWING THE RELATIVE MULTIPLICATIONS OF PARATYPHOSUS A AND B STRAINS. NEUTRAL MEAT EXTRACT PEPTONE BROTH AT 37 C. USED AS NUTRIENT

	Initial Number Per Cc	After 3 Days	After 7 Days
Para B strain 12................	9,400	209,000,000	140,000,000
Para B strain 180................	14,000	540,000,000	105,000,000
Para A strain 158................	7,800	14,000,000	70,000,000
Para A strain 219................	9,600	81,000,000	90,000,000

In rate and amount of multiplication in milk, therefore, No. 221 occupies an intermediate position between the common types of paratyphoid A and paratyphoid B bacilli. This corresponds exactly with its relatively tardy alkali production.

The Para A strains also appear to multiply in nutrient broth (Erlenmeyer flasks of 50 c c) more slowly than the Para B strains (Tables 5 and 6).

Flasks of nutrient broth incubated at 20 C. instead of 37 showed a similar difference in the rate of multiplication between the A and B

TABLE 6

COMPARISON OF THE RAPIDITY OF THE MULTIPLICATION OF STRAINS THE NUTRIENT BROTH WAS NEUTRAL MEAT EXTRACT PEPTONE AT 37 C.

	Initial Number Per Cc	After 24 Hours	After 48 Hours	After 72 Hours
Para B strains (5, 8, 299) Average .... (2 plates each flask)	19,200	540,000,000	430,000,000	340,000,000
Para A strains (3, 9, 131) Average .... (2 plates each flask)	11,500	123,000,000	133,000,000	108,000,000

types although the total figures reached were not so great. The addition of 0.1% dextrose to the nutrient broth did not noticeably change the numerical relations of the two types. The rate of multiplication of the Para A strains was still less rapid than that of the B strains.

The differences between the Para A and Para B types in rapidity of alkali formation in milk seem from these facts to be largely, if not altogether, a numerical relation due to gradations in the amount and rate of multiplication.

THE INFLUENCE OF AN OXIDIZING SUBSTANCE (SODIUM IODOXYBENZOATE) ON THE CATALASE VALUE OF THE BLOOD AND TISSUES

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Sodium iodoxybenzoate is an organic peroxid. It is an iodin substitution product of orthoamido-benzoic acid containing two atoms of oxygen bound to the iodin molecule. The method of preparation of this substance and the determination of its available oxygen have been described by Loevenhart and Grove[1] and by Arkin.[2]

The pharmacology of this substance was first studied by Loevenhart and Grove[1] in the hope of obtaining some experimental evidence as to the mechanism of physiologic oxidation. Iodoxybenzoic acid and its salts seemed to be especially suited to this purpose because they can be used for intravenous injection. They found that sodium iodoxybenzoate very readily oxidizes hemoglobin to oxyhemoglobin, showing that the oxygen contained in the molecule is available for physiologic oxidation in the same manner as the oxygen taken up in the lungs. Experiments tending to show the availability of the oxygen for oxidation by the tissues were not conclusive, since sodium iodoxybenzoate was unable to furnish the oxygen necessary for the peroxid reaction. On the other hand, depression of the respiratory center and the production of long periods of apnea indicate the physiologic activity of the oxygen on the respiratory center. Following intravenous injection, there is a moderate leukocytosis which consists for the most part in an increase in the polymorphonuclear leukocytes.

The bactericidal action of sodium iodoxybenzoate has been studied by Arkin,[3] who found that it possesses a marked bactericidal action for B. typhosus, B. pyocyaneus, B. coli and Staphylococcus aureus. Moreover, its strength as a bactericide was related to its oxidizing power. In experiments with B. typhosus, sodium iodoxybenzoate proved to be 200 times as bactericidal as sodium iodbenzoate and twice as bactericidal as sodium iodosobenzoate. Sodium iodbenzoate contains no available oxygen, while sodium iodosobenzoate contains but one molecule of available oxygen or about one-half the available oxygen of iodoxybenzoate.

Sodium iodoxybenzoate in its relation to the biologic reactions has been studied by several authors. Hektoen[4] found that dogs which had been injected with goat blood followed by an intravenous injection of sodium iodoxybenzoate

[1] Jour. Pharmacol. and Exper. Therap., 1911, 3, p. 101.
[2] Jour. Infect. Dis., 1913, 13, p. 408.
[3] Jour. Pharmacol. and Exper. Therap., 1911, 3, p. 145.
[4] Tr. Chicago Path. Soc., 1911, 8, p. 138.

produced more lysin than dogs receiving only goat blood, and suggests that the production of antibodies is related to physiologic oxidation. It is interesting to note that a single injection proved quite as effective as repeated injections. Amberg and Knox[5] have shown that the intravenous injection of iodoxybenzoate always markedly lessens the intensity of the intracutaneous reaction in rabbits sensitized with horse serum, in some cases resulting in a complete absence of reaction. This effect proved to be only temporary, lasting but a few days. These results furnish evidence that a physiologically active oxidizing agent decreases the intensity of a local inflammatory reaction. Additional evidence in support of this is the observation of Amberg[6] that the intravenous injection of sodium iodosobenzoate diminishes the inflammatory reaction produced by the sub-cutaneous injection of mustard oil and diphtheria toxin in rabbits.

A series of experiments to determine the effect of sodium iodoxybenzoate on the production of immune bodies and related reactions has been carried out by Arkin. Sodium iodoxybenzoate markedly increased the phagocytic activity of leukocytes in vitro,[7] and resulted in an increased production of specific hemolysin for ox corpuscles and agglutinins for the typhoid bacillus when injected into immunized rabbits.[8] It was also found to have an inhibitory effect on the local anaphylactic reaction in tuberculous guinea-pigs. These experiments show a definite relation between physiologic oxidation and the immune reactions, since a substance which contains physiologically active oxygen stimulates the production of immune bodies. Arkin has suggested that the influence of sodium iodoxybenzoate is exerted not through the liberation of the oxygen contained in the molecule, but that it stimulates the tissues which are the site of antibody formation in the manner of a catalytic agent.

Catalase is an enzyme belonging to the class of oxidases, which possesses the specific property of decomposing hydrogen peroxid with the liberation of molecular oxygen. This ferment was first isolated and named by Loew[9] in 1901. He was able to demonstrate its specificity and almost universal occurrence in plant and animal tissues. A. Schmidt[10] demonstrated that the catalase of the blood is contained almost exclusively in the red blood corpuscles, and Bergengrün[11] later proved that the stroma and not the hemoglobin was the active agent.

CATALASE OF THE BLOOD

According to Winternitz[12] the catalase value of the blood of a normal rabbit is constant from day to day, showing only such changes as come within the limits of error of the method. It differs quite markedly, however, in different individuals of the same species. Rab-

5 Jour. Pharmacol. and Exper. Therap., 1911, 3, p. 223.
6 Ztschr. f. d. ges. Exp. Med., 1913, 2, p. 19.
7 Jour. Infect. Dis., 1912, 11, p. 427.
8 Jour. Infect. Dis., 1915, 16, p. 349.
9 Report 68, U. S. Dept. Agric., Wash., D. C., 1901.
10 Arch. f. d. ges. Physiol., 1872, 6, p. 413.
11 Inaug. Diss., Dorpat, 1888.
12 Jour. Exper. Med., 1909, 11, p. 200.

bits from the same litter and similar in size and development are more nearly alike in their catalase activity than those varying in size and age. Fully developed and well nourished rabbits showed a much higher catalase activity of the blood than young and poorly developed animals. Strauss[13] reports that starvation is accompanied by a rise in catalytic activity of the blood of rabbits which returns to a lower level when feeding is resumed.

Very little work has been done to determine directly the effect of oxidation on the catalase value of the blood. Complete thyroidectomy in rabbits is followed by a marked and permanent decline in the catalytic activity of the blood, according to Winternitz and Pratt.[14] In incomplete thyroidectomy the fall, if any occurs, is only temporary. Feeding thyroid extract to thyroidectomized animals gradually raises the catalytic activity of the blood to the normal level, but never goes beyond even with excessive feeding. Thyroid extract when fed to normal rabbits did not influence their blood catalase. On the other hand Burge, Kennedy and Neill[15] report that feeding thyroid to the cat increases the catalase value of its blood and suggest that this increase in catalase of the blood may account for the increased oxidation during thyroid feeding.

Experiments.—Our experiments were undertaken as the first step in an attempt to analyze the mechanism by which sodium iodoxybenzoate, a physiologically active oxidizing agent, affects the biologic reactions. The function of catalase is still unknown, although its universal occurrence has led to the belief that it must play an important rôle in the physiologic economy of the organism. Since the only known property of catalase outside the body is its ability to decompose hydrogen peroxid, several theories have assigned to it an important rôle in physiologic oxidation. There was thus the additional possibility of obtaining some information as to the function of catalase.

Rabbits were used in pairs as nearly as possible alike in color, age and state of nutrition. The method of determining the catalase value of the blood was essentially that of Winternitz.[16] Blood was obtained from the ear and drawn into a fine pipet graduated to contain 0.025 c c. This was immediately diluted with 10 c c of distilled water, making a dilution of 1 : 400. Five c c were then placed into each of two 100 c c salt mouth bottles. A small vial containing 5 c c of hydrogen peroxid was introduced into one of the bottles which was then connected with a gas buret. After a little practice the small vial could be overturned

[13] Bull. Johns Hopkins Hosp., 1912, 23, p. 51.
[14] Jour. Exper. Med., 1910, 12, p. 115.
[15] Am. Jour. Physiol., 1917, 43, p. 433.
[16] Arch. Int. Med., 1911, 7, p. 624.

by a single violent shake. The bottle was vigorously shaken for 1 minute to insure thorough mixing. Readings were taken after 1 minute and at intervals of 30 seconds for 3 minutes. Both samples were usually titrated and the average number of c c of oxygen liberated after 3 minutes recorded as the final result. The hydrogen peroxid used was Mallinckrodt's containing 3%. Each bottle was titrated for acidity and neutralized before use. Because of slow deterioration it was found necessary to make frequent titrations of the hydrogen peroxid by means of lead peroxid as described by Winternitz.[10]

TABLE 1

Catalase Content of Blood of Rabbits Injected with Salt Solution (A) and N/20 Sodium Iodoxybenzoate Solution (B) (See Chart 1)

Date	C c of Oxygen in 3 Minutes	
	Rabbit A	Rabbit B
Before injection:		
9-16	14.8	17.2
9-17	17.2	23.0
9-18	16.0	17.0
9-19	20.2	19.8
9-22	14.5	19.0
9-23	16.3	18.7
9-24	16.1	16.9
9-25	15.9	15.6
9-27	16.9	16.8
9-28	18.1	17.4
9-29	16.5	18.1
9-30	16.1	17.1
10- 5	12.0	13.0
10- 6	10.4	10.0
10- 7	16.5	20.3
10- 9-11 a. m.	21.0	23.0
10- 9- 4 p. m.	19.5	23.7
After injection:		
10- 9- 7 p. m.	15.1	20.0
10-10	13.6	16.7
10-11	19.4	21.2
11-12	18.4	18.8
10-13	17.3	19.7
10-15	16.2	18.5
10-16	15.4	20.2
10-19	17.7	21.3
10-21	18.4	20.7
10-29	15.6	16.3
11- 6	17.5	20.4
11-16	19.2	22.6
11-21	19.5	20.8

Table 1 contains the data on the daily variation of the catalase activity of a pair of rabbits before and after injection. Rabbit A served as control and was injected with 5 c c of sterile salt solution, while Rabbit B was injected with 5 c c of neutral N/20 sodium iodoxybenzoate solution. The control rabbit showed a daily variation ranging from 10.4 c c, the lowest, to 21 c c, the highest, or a maximum difference of 10.6 c c. Following the injection of salt solution the greatest variation was 5.9 c c. The catalase value of the blood of Rabbit B before injection varied from 10 c c-23.7 c c, giving a difference of 13.7 c c

under normal physiologic conditions.   Two and one-half hours after
the injection of sodium iodoxybenzoate there was a fall of 3.7 c c which
increased within 24 hours to 7 c c.   These differences lie well within
the range of normal physiologic variation.   When plotted graphically
(Chart 1) the curves of catalase variation in the two animals before
and after injection show no marked differences.   Several series of
animals were treated in the same way over a shorter period of time
with the same results.   Other experiments gave the same results.
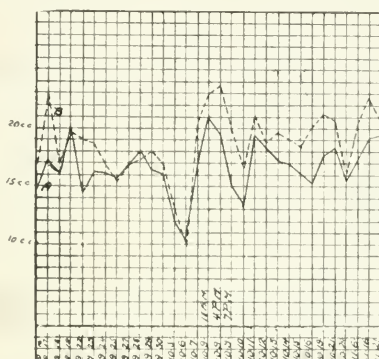
Chart 1.—Showing the daily variation in catalase content of the blood before and after
injection. Both animals injected 4:30 p. m., 10/9/14 (note Table 1).

CATALASE OF TISSUES

Battelli and Haliff[17] in a study of the catalase value of the tissues
of various species of animals found that the tissues of an animal show
quite marked differences in catalytic activity.   The same tissue from
animals of different species shows a difference in activity, but within
a given species the same organ possesses a constant catalase value
among the individuals of that species.   The catalytic activity was not
in proportion to body temperature, since cold blooded animals such
as the adder have tissues richer in catalase than many warm blooded
animals such as the rabbit.   In a general way the catalytic value of the
tissues of the various animals studied in the order of decreasing activity
was as follows: liver, kidney, blood, spleen, lung, heart, muscle, and
brain.   In most species the liver possesses the greatest catalytic activity.
The kidney of the rabbit is slightly richer in catalase than the liver.

[17] Compt. rend. Soc. de biol., 1904, 2, p. 264.

Spitzer,[18] by comparing the power of tissues to decompose hydrogen peroxid with their power to oxidize salicylic aldehyd noted a similarity in the two cases, and concluded that the power of tissues to decompose hydrogen peroxid is a true measure of their oxidizing power. Spitzer's work has been questioned by Battelli and Stern[19] who point out that his methods were faulty and not based on a quantitative determination of active catalase. Moreover, he assumed that the substance which actively decomposes hydrogen peroxid is identical with the other oxidases, and that this reaction is of the same type as all fermentative oxidations. Subsequent work has demonstrated that catalase is a specific enzyme. Kastle and Loevenhart[20] found that HCN strongly inhibits the action of liver catalase on hydrogen peroxid, thus showing that a substance which interferes with physiologic oxidation also interferes with the action of catalase. Using corresponding muscles for comparison, Burge[21] found a greater catalytic activity in warm blooded animals in which oxidation is more intense than in cold blooded animals. The amount of catalase in the different muscles of the body varies with the amount of work which they are normally called on to perform, and in any single muscle increases with an increase in external physical work. It would seem, therefore, that the catalase content of a muscle is directly proportional to the amount of oxidation in that muscle.

Experiments.—Since we were unable to demonstrate any change in the catalase value of the blood of rabbits following the intravenous injection of sodium iodoxybenzoate, our next problem was to determine its effect on the catalase value of the tissues.

Here again, the method is essentially that of Winternitz.[22] Rabbits were used in all experiments. The organs were rendered as nearly blood free as was possible without washing them, by bleeding the animals from both carotids. Ten gm. of the fresh organ were ground in a mortar with washed white sand. To this was gradually added 20-30 c c of distilled water and the mixture strained through clean cloth. The residue was ground again, distilled water added, and strained as before. This was repeated once more and the combined filtrates diluted with distilled water to 100 c c, making a 10% aqueous extract. In the case of organs weighing less than 10 gm., the whole was ground up with enough water to make a 10% emulsion. One c c of the emulsion to be tested was diluted to 5 c c with distilled water in a 100 c c salt mouth bottle, hydrogen peroxid added and the catalase value determined as previously described. In our preliminary experiments we used 5 c c of neutralized hydrogen peroxid, but later

[18] Quoted by Kastle and Loevenhart.[20]
[19] Arch. di Fisiol., 1905, 2, p. 471.
[20] Am. Chem. Jour., 1903, 29, p. 397.
[21] Am. Jour. Physiol., 1916, 41, p. 153.
[22] Jour. Exper. Med., 1908, 10, p. 759.

experience showed this to be insufficient for the organs rich in catalase. In the experiments reported 10 c c of neutralized Mallinckrodt's hydrogen peroxid were used. We also found it necessary to standardize our hydrogen peroxid against lead peroxid each time before use. Duplicate determinations were always made and the tabulated results represent the average number of c c of oxygen liberated by the action of 0.2 c c of a 10% emulsion on 10 c c of 3% hydrogen peroxid in 3 minutes.

Table 2 contains the results of the determinations of the catalase value of the kidney, liver, spleen, lungs and muscle for six normal rabbits in a decreasing order of activity. We find, as did Battelli and Haliff, that in the rabbit the kidney shows the highest catalytic activity,

TABLE 2

Catalase Value of Organs of Normal Rabbits*

Kidney	Liver	Spleen	Lungs	Muscle
105.5	110.4	68.8	30.8	2.4
105.3	100.5	35.0	20.2	1.9
95.0	85.6	27.8	25.6	2.4
104.5	81.9	18.0	23.3	2.4
70.0	67.9	26.3	16.8	
83.6	85.3	35.3	22.5	

*C c of oxygen in 3 minutes, using 0.2 c c of 10% emulsion and 10 c c of neutral hydrogen peroxid.

TABLE 3

Catalase Value of Organs of Rabbits Injected with Sodium Iodoxybenzoate*

	Kidney	Liver	Spleen	Lungs	Muscle
Two animals killed 1 hour after injection	104.5 99.5	76.2 86.2	42.8 67.4	41.2 31.7	1.8 2.4
Two animals killed 24 hours after injection	106.0 99.3	111.0 73.6	50.8 51.9	40.2 21.4	3.0 2.4
Two animals killed 10 days after injection	100.0 98.6	76.4 99.5	42.3 32.5	19.8 26.7	2.2 2.0

*C c of oxygen in 3 minutes using 0.2 c c of 10% emulsion and 10 c c of neutral hydrogen peroxid.

being slightly more active than the liver except in one animal. Of all the tissues tested, skeletal muscle was the lowest in activity and the most constant. The catalase values of the tissues of the individual animals show considerable differences, but we have noted that the more nearly the animals are alike as to color, size and general state of nutrition the more closely does the catalase value of their tissues agree.

Table 3 shows the catalase value of the tissues of rabbits injected with 5 c c of neutral N/20 sodium iodoxybenzoate solution intra-

venously, and killed at varying intervals following injection as indicated in the table. None of the organs shows values markedly differing from those of the normal animals, which might not be accounted for as differences between individuals of the same species. The spleen has a rather high value in the injected animals, but no definite conclusions can be based on this because of the wide variations among normal animals.

CONCLUSIONS

The catalase value of the blood of normal rabbits may show considerable daily variation under normal conditions, and is not as constant as previous experiments would seem to indicate. Any change in catalase content due to experimental conditions must therefore be excessively high or excessively low before it can be attributed to such conditions.

The intravenous injection of sodium iodoxybenzoate in normal rabbits has no effect on the catalase value of the blood, that might not be accounted for as a normal physiologic variation.

We have found rather marked variations in the catalase activity of tissues of normal rabbits.

Rabbits injected intravenously with sodium iodoxybenzoate do not show catalase values of their organs markedly higher or lower than those occurring among normal rabbits. A possible exception is the spleen which shows an increase in catalytic activity. However, this increase is not greater than might be accounted for on the basis of individual variation.

OBSERVATIONS ON MENINGOCOCCUS CARRIERS AND ON THE BACTERIOLOGY OF EPIDEMIC MENINGITIS

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Because epidemic meningitis is especially prone to occur in military camps, the problems relating to the control of this disease are of particular interest at this time. The exact conditions under which this disease becomes epidemic have not been determined. It seems probable that the number of susceptible individuals in any community is very small and that crowded living quarters, fatigue, exposure, and frequent changes in the population are factors which favor the development of the infection. It is the accepted view that the disease is spread largely by direct contact with healthy persons harboring meningococci in the nose and throat rather than by patients. Accordingly, at the present time, the most important procedure relied on for the control of epidemic meningitis involves the identification and detention of healthy meningococcus carriers. The weight of experience so far at hand favors this procedure, but more evidence is needed to determine its exact value. Such evidence must necessarily come from painstaking bacteriologic investigations of epidemics of meningitis. Recently we have been engaged in the study of this disease in a large military camp. In this work bacteriologic examinations were made of more than 15,000 men, and a number of meningococcus carriers were identified. In an extended study of these carriers certain observations have been made which are of sufficient interest to be recorded. Furthermore, a large number of strains of meningococci have been obtained, both from the nasopharynx of carriers, and from the spinal fluid of infected individuals in different localities. A careful comparative study of the cultural and biologic characters of these organisms have been carried out and the results are included in this article.

BACTERIOLOGIC TECHNIC WITH SPECIAL REFERENCE TO
CARRIER DETECTION

At the beginning of this work it was necessary to develop a technic for the identification of the meningococcus which was simple, reliable, and practical. In our hands the technic herein described fulfils these requirements.

Since in the carrier the meningococcus most frequently inhabits the secretions of the posterior nares and nasopharynx, cultures must be made from these sources. In our experience the necessary material is obtained best by means of a simple swab made by wrapping cotton about the end of a piece of stovepipe wire about 25 cm. long. The straight swabs are sterilized in the autoclave in test tubes and then bent near the ends over the edge of the tube to an angle of 45 degrees as each is withdrawn for use. To make the smear the tongue is depressed, and during phonation the swab is passed behind the soft palate and a thorough smear of the posterior nasopharyngeal surfaces made; as the swab is removed care is taken not to touch its posterior surface against the throat or tongue. A small portion of material on the posterior surface of the swab is immediately placed on the surface of the plated culture medium, near the edge of the plate, and streaks are made from this material in various directions with a sterile wire needle. When possible only one culture should be made on a plate and the plate should be placed in the incubator without delay; a few hours at ordinary summer temperature however does not seem to affect the results appreciably, but in colder weather the plates must be warmed to 37 C. and packed in suitable containers if they are to be transported even comparatively short distances without danger. The plate cultures previously warmed to 37 C. should not be kept out of the incubator longer than 1 hour after inoculation. The swabs are flamed or dipped in a strong disinfectant after using.

Careful comparison of a large number of culture mediums recommended for this work has been made, and we find that plain blood agar gives the most satisfactory results. It is a medium that is easily prepared and at present everywhere easily obtained in this country. Bottles or flasks with 100-500 c c of plain agar are handy for this work. Defibrinated blood, either human, horse, goat or sheep, is easily procured and small amounts suffice for a large number of plates. After melting the agar in the flask or bottle it is cooled down to 45-47 C. when blood is added in a proportion of 5-10%. It is well to warm the blood to about 40 C. before adding it to the agar. After mixing, the blood agar is poured into sterile petri dishes and allowed to cool. By incubation, tests for sterility of the medium are made.

The meningococcus is a gram-negative diplococcus. Other members of the group of gram-negative diplococci, except the gonococcus, frequently inhabit the posterior nasopharynx and the differentiation of the meningococcus from these other gram-negative diplococci is a most important step in carrier detection. On blood agar the meningococcus colonies vary in size, are very moist, semitranslucent, elevated, sticky in consistency, and of indistinct outline; often colonies coalesce in irregular masses. When viewed in transmitted light the colonies have a faint bluish gray tint and are almost transparent. Micrococcus catarrhalis cannot be differentiated from the meningococcus by the appearance of the colonies in blood-agar plate cultures, although the catarrhalis colonies are often larger and more opaque than the meningococcus colonies. According to our

experience growth at room temperature or the use of transparent culture medium has little practical value in the differentiation of the meningococcus and Micrococcus catarrhalis. Here we would state that the use of blood agar has a great advantage over transparent mediums in that the members of the streptococcus-pneumococcus group are easily differentiated from the meningococcus by the appearance of the colonies. This is an important point for these organisms are often found in cultures and the colonies resemble meningococcus colonies on transparent mediums. In the instance of Micrococcus pharyngis siccus and Micrococcus flavus the characteristics of the colonies are such that the 24-hour culture on blood-agar plates usually suffices for differentiation from the meningococcus. Micrococcus pharyngis siccus colonies are medium sized, flat, grayish in color, dry, and often wrinkled on the surface. The flavus group of organisms in young cultures resemble the meningococcus in the appearance of their colonies. After 24-36 hours' incubation, however, the colonies become yellowish in color, opaque, somewhat dry, and pointlike in consistency. Colonies of the flavus group on blood-agar plates can usually be identified by these characteristics, but in some instances agglutination tests are necessary for the final diagnosis of the organism.

The blood-agar plate cultures of the nasopharyngeal material are examined after 16-24 hours' incubation and the suspicious colonies transferred to blood-agar slants. It is important that all tubes used in meningococcus cultures be warmed to 37 C. before inoculation. The subcultures are incubated for 8-16 hours at 37 C. and used for identification tests. In making nasopharyngeal cultures time can be saved by taking the swabs in the late afternoon, incubating the cultures overnight; transfers made from the suspicious colonies on the following morning, grow out sufficiently for agglutination tests the same afternoon. This procedure makes it possible to report the final results of cultures in 36 hours. The original plates are set aside at room temperature and the suspicious colonies again examined 12-24 hours later for the development of pigment.

For agglutination tests polyvalent antimeningococcus serum of high potency is used in dilutions of 1/50, 1/100, 1/500; also normal horse serum in a dilution of 1/50 for control. Equal parts of the serum and a suspension of the bacteria in salt solution (the growth from an ordinary 24-hour blood-agar slant culture of meningococci in 3 or 4 c c of normal salt solution) are mixed and incubated for 6-12 hours at a temperature of 55 C. If suspensions of a given coccus are agglutinated in high dilutions with the antimeningococcic serum, and not by the normal serum, the organism is treated as a meningococcus, and the person from whom the culture was obtained is isolated as a carrier. The microscopic test devised by Tunnicliff[1] is also reliable and adapted to more rapid diagnosis.

A satisfactory medium for fermentative tests is the Hiss serum water containing litmus as an indicator and 1% of the test sugar. The fermentative reactions of the gram-negative diplococci concerned are such that glucose and saccharose are the only sugar necessary as a rule. The meningococcus ferments glucose and not saccharose; Micrococcus catarrhalis does not change either sugar; while the other members of this group occurring in the nasopharynx change both glucose and saccharose. As a measure of economy the sugar mediums are put up in amounts of 2 c c in small test tubes 1 cm. in diameter. Transfers are usually made to the sugar mediums from the first subcultures from the suspicious colonies. The sugar medium cultures are tested for growth after

[1] Jour. Am. Med. Assn., 1917, 69, 786.

24 hours' incubation. These cultures however must be incubated 5-7 days before final readings, and great care must be exercised to avoid contamination. The fermentative reactions coincide with the results of the agglutination tests as a rule.

In making cultures of cerebrospinal fluid the sediment of a centrifugalized specimen of freshly drawn fluid is planted on blood-agar slants as well as in tubes of 1% dextrose broth containing serum or ascites fluid. In the instances in which the spinal fluid does not yield a sediment on centrifugalization the whole fluid is planted in 1% dextrose broth containing serum or ascites fluid. The broth cultures of spinal fluid should be incubated from 3-5 days before they are discarded. Using these methods meningococci were isolated from the spinal fluids of meningitis patients in practically all instances. The cultural characteristics of each organism as well as the biologic characters are determined in each instance. The stock cultures are kept on defibrinated blood agar at room temperature. Transfers are made every 24-48 hours to insure the life of the culture.

RESULTS

As soon as a case of meningitis occurred the immediate contacts were examined bacteriologically, the carriers identified and isolated. If more than one case occurred in an organization, the entire organization was examined for carriers. It was found that 3-6% of the men examined harbored meningococci in the secretions of the nose and throat. The number of carriers in different groups of men varied (Table 1). One hundred and forty immediate contacts (tent-

TABLE 1

Summary of Examinations for Meningococcus Carriers

Source of Material Examined	Number Examined	Percentage
Convalescents from epidemic meningitis................	38	38.2
Hospital corps	46	13.5
Immediate contacts	140	26.7
Other members of organizations in which epidemic meningitis occurred	15,257	4.4
Total number of persons examined.............. 1.2% were found to be chronic carriers	15,351	

mates of cases of meningitis) were examined, in most instances within 12 hours after the case of meningitis was found; 26.7% of these were found to be meningococcus carriers. Seven of the immediate contacts, or 5%, proved to be chronic carriers. Thirty-eight patients, convalescent from the disease, were cultured and 38.2% were identified as carriers. All were discharged, however, in a few weeks with negative cultures. At the time of the epidemic 13.5% of the hospital attendants yielded positive meningococcus cultures. In this group the

nurses taking care of the meningitis cases were included. Of other men, members of organizations in which cases of meningitis had occurred, 15,257 in all, 4.4% yielded positive meningococcus cultures. Of all the persons examined 1.2% proved to be chronic carriers.

It seems important at this point to emphasize the distinction between two tyes of meningococcus carriers. The chronic carriers, individuals yielding positive nasopharyngeal cultures over long periods of time, were few in number and only in rare instances developed meningitis. In the cultures from these persistent carriers the meningococci in most cases were present in predominating numbers; indeed many pure cultures were obtained in such cases. In our experience the connection between chronic meningococcus carriers and cases of meningitis has been striking in many instances, and seem to us to indicate the necessity of special attention being given to this type of carrier in all epidemics of this disease. On the other hand the temporary carriers were more numerous and in the cultures from these individuals the number of meningococci was small as a rule. These persons seem to harbor meningococci in their nasopharynx for a short period of time only.

During the course of this work repeated bacteriologic examinations have been made of the interns, nurses, and attendants coming in contact with cases of epidemic meningitis in the hospital of the Memorial Institute, and no meningococcus carriers have been found. It seems reasonable to regard this result as due in large degree to the use of the precautionary measures, including the wearing of face-masks, described by Weaver.[2]

A point or two in regard to the bacteriologic examination of meningococcus carriers may be emphasized here. The intricacy of the bacteriologic methods involved and the anatomy of the nasopharyngeal structures make the detection of the meningococcus carrier a rather difficult procedure. A certain small number of carriers especially those persons who have only a few menigococci in the nasopharyngeal secretions no doubt are overlooked. Furthermore one or two negative cultures may be obtained from chronic carriers who yield positive cultures on subsequent examination. The taking of the swab should be as thorough as possible for the meningococcus seems to grow in isolated foci in the upper respiratory tract.[2a] Before a chronic carrier

[2] Jour. Am. Med. Assn., 1918, 70, p. 76.
[2a] Herrold, R. D.: Jour. Am. Med. Assn., 1918, 70, p. 82.

is discharged from isolation it seems advisable to obtain at least 3 successive negative cultures. Perhaps these persons should be under close observation for a time even after they return to their organizations.

BIOLOGIC REACTIONS OF MENINGOCOCCI FROM DIFFERENT SOURCES

The classification of meningococci on the basis of biologic differences was first attempted by Dopter[3] in 1909. Dopter isolated organisms from the nasopharynx of contacts which resembled meningococci in every characteristic except serum reactions. He also, a little later, demonstrated these atypical organisms in the spinal fluid of cases of meningitis, and on the basis of biologic differences he established two main groups—meningococcus and parameningococcus. Dopter's work has been confirmed and extended by many investigators, particularly Wollstein[4] in America, Gordon,[5] Arkwright,[6] Ellis,[7] Fildes and Baker,[8] and others in England, Nicolle,[9] and others in France. From these observations it appears that the meningococci fall into two large biologic groups: The typical meningococcus (Group 1) and the parameningococcus (Group 2). A great variety of intermediate strains related to one or the other of the two large groups occur, however, and Gordon has classified these intermediates in 2 groups which he calls Groups 3 and 4. Biologic differences in meningococcus strains are of great practical importance in the identification of the organism and in the preparation of good therapeutic serum. Moreover, problems relating to the meningococcus carrier can be studied more efficiently on the basis of such differentiation.

By the use of biologic reactions we have been able to compare meningococci obtained from cases of meningitis and from carriers in the same and different localities with interesting results.

In this phase of the work monovalent serum of high potency was prepared for known pathogenic strains of meningococci, that is, organisms isolated from the spinal fluids in cases of meningitis. In the preparation of serum young rabbits weighing 1,500-2,000 gm. were used, and a monovalent serum having an agglutination titer of 100-300

[3] Compt. rend. Soc. de biol., 1909, 67, p. 74.
[4] Jour. Exper. Med., 1914, 20, p. 201.
[5] Jour. Royal Army Med. Corps, 1915, 25, p. 411. Also Medical Research Committee, Special Report, Series No. 3, 1917.
[6] Brit. Med. Jour., 1915, (2) p. 885.
[7] Ibid., p. 881.
[8] Lancet, 1918, 194, p. 92.
[9] Bull. et mém. de la Soc. méd. d. Hôp., 1917, 41, p. 878.

or higher for its homologous organism was considered satisfactory for the experiments.

In the agglutination tests unheated monovalent serum was used in dilutions of 1/50, 1/100, 1/200, 1/300, 1/400, 1/500, 1/600, 1/700, 1/800, 1/900 and 1/1000, and normal rabbit serum in dilutions of 1/50 and 1/100 for controls. The bacterial suspensions were prepared by suspending the growth from one 24-hour blood-agar slant culture of the organism to be tested in 4 c c of sterile normal salt solution. By this means moderately turbid suspensions were obtained. Equal amounts, usually 0.25 c c, of bacterial emulsion and the different dilutions of immune and normal serum were mixed, and incubated for 24 hours at 55 C. A positive agglutination was recorded only in those instances in which agglutination was complete. In all the agglutination tests the macroscopic method was used.

TABLE 2

Biologic Reactions of Meningococci Obtained from the Spinal Fluid of Cases
of Meningitis

Strain of Meningococcus	Serum											
	2406	2425	2379	2385	2363	2457	2353	2372	A	B	C	D
2406	400	800	700	100	400	1000	500	200	200	0	300	0
2425	800	900	600	200	500	400	500	600	400	0	800	0
2379	1000	1000	500	100	300	1000	1000	300	1000	0	500	0
2385	1000	200	1000	400	1000	1000	900	1000	500	500	500	200
2363	300	500	500	200	900	600	300	300	300	0	400	0
2457	1000	1000	700	50	800	300	700	500	200	0	500	0
2353	1000	1000	800	100	500	500	600	400	100	0	300	0
2372	800	1000	800	300	800	400	700	300	500	0	400	0
A	1000	1000	700	200	500	1000	1000	400	500	0	300	0
B	0	0	0	0	0	0	0	0	0	500	100	600
C	700	1000	900	200	1000	1000	400	800	800	100	1000	100
D	0	0	0	0	0	0	0	0	0	0	0	10?

Using these methods 36 strains of meningococci from the cerebrospinal fluid of cases of meningitis and 114 strains from the nasopharyngeal secretions of chronic carriers have been examined. All of these organisms had the morphologic and cultural characters of the meningococcus and were in most instances agglutinated by a polyvalent antimeningococcus serum. In a series of cross agglutinations using 12 of the pathogenic strains of meningococci from sporadic cases of meningitis occurring in Chicago, and their homologous immune serums, 2 biologic groups were demonstrated. In one group there were 2 and in the other group 10 strains.

The results of these tests are recorded in Table 2. In the initial experiments the agglutination titer of the monovalent serums for the heterologous strains varied, and in a few instances cross agglutination

between the two groups occurred. These variations in agglutinability, however, did not seem of sufficient degree to warrant more complex biologic division. One of the groups corresponds to the typical meningococcus, and the other to the parameningococcus, according to cross agglutination tests using strains of these organisms obtained from the Laboratory of the United States Public Health Service, Washington, D. C.

The biologic characters of strains of meningococci from cases of epidemic meningitis and chronic carriers occurring in Chicago, and in the armed camp during the epidemic, as determined by agglutination tests with representative serum of high titer for each of these biologic groups have been studied (Table 3).

TABLE 3

Biologic Classification of Meningococci from Different Sources as Determined by Agglutination

Source of Strains	Group 1		Group 2	
	No. of Strains	Per cent. of Total	No. of Strains	Per cent. of Total
Cerebrospinal fluid, meningitis. Military camp	12	85.7	2	14.3
Nasopharynx of carriers. Military camp	95	83.3	19	16.7
Cerebrospinal fluid, meningitis. City of Chicago	3	13.7	19	86.3

All of the strains of meningococci so far studied, except 12, were agglutinated by one or the other of the type serums. Of the 36 strains obtained from cases of meningitis 22 were from sporadic cases occurring in Chicago. Nineteen of these, or 86.3%, were found to belong to Group 2. Furthermore in two instances in which the contacts were examined, carriers were found which harbored organisms of the same biologic type. On the other hand, the meningococci isolated from cases of meningitis and chronic carriers in the military camp were found to belong to Group 1 in most instances. Of the case strains 85.7% and of the carrier strains 83.3% were classified in Group 1. The interesting fact emerges from this summary that the prevailing types of meningococcus causing meningitis in the two localities differed in their biologic reactions. But the organisms isolated from cases of meningitis and carriers in the same locality were similar in biologic reaction in the majority of instances.

In the agglutination experiments marked differences were observed in the agglutinability and agglutinogenic properties of certain strains of meningococci. Of the 150 strains examined 121 yielded specific group reactions and were classifiable in one or the other of the 2 groups. Twenty-nine strains were irregular in their biologic reactions. Seventeen of these atypical strains were agglutinated by representative serums of both groups. In all instances, however, the dilution of serum in which agglutination of these organisms occurred was much higher with one group than the other. These meningococci were classified in the group represented by the serum with the highest agglutinin content for the particular strain. To gain further information of the biologic reactions of these irregular strains monovalent serum was prepared from 4 of the 17 irregular organisms. These monovalent serums, however, yielded specific reactions with meningococci of one or the other of the two main groups as indicated by the results of the original agglutination tests. The remaining 12 of the atypical strains were not agglutinated by the group monovalent serums or the polyvalent serums. Eleven of these inagglutinable strains were obtained from carriers and one from the cerebrospinal fluid of a patient with meningitis. All of these organisms revealed the typical cultural and morphologic characters of the meningococcus. Monovalent serums prepared from these strains agglutinated in a specific way meningococci belonging to one or the other of the large biologic groups. In this manner it has been possible to classify all the inagglutinable strains so far encountered (See Diagram).

DIAGRAM SHOWING GROUPING OF MENINGOCOCCI

MENINGOCOCCI

Group 1   Group 2

Typical   Atypical   Inagglutinable   Typical   Atypical   Inagglutinable

The atypical subgroups includes strains with variable agglutinative reactions and tendency to cross agglutination.
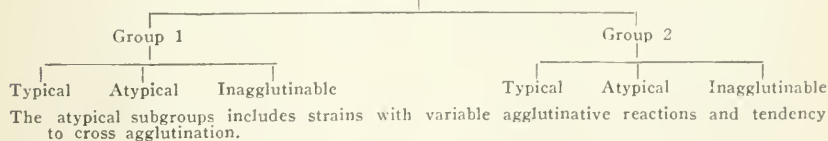
It is interesting to note that the biologic reactions of the carrier strains of meningococci are atypical in more instances than those of the case strains. Furthermore, differences in agglutinability were observed among meningococci belonging to the same group.

Clinically certain Group 2 (parameningococcus) infections have not reacted to treatment with polyvalent serum of high potency. These cases run a chronic course and terminate fatally after a few

weeks. It seems probable that a monovalent Group 2 serum might be efficacious in such cases.

Meningococci with biologic characters similar to those of the atypical strains met with in this work have been the subject of much discussion. Some investigators have been content to call them intermediates without attempting to classify them. Gordon and his associates have classified these variants into two biologic groups (Groups 3 and 4). The importance of these debatable biologic groups, however, seems more apparent than real for they are closely related to the two main groups.

The results of the immunologic observations described herein suggest that the meningococcus can be divided into two large groups on the basis of biologic reactions which correspond to the meningococcus and parameningococcus as described by Dopter and others. The members of these large groups, however, vary in their agglutinability and agglutinogenic properties within wide limits, and include typical, irregular and inagglutinable organisms.

SUMMARY

During the summer and fall of 1917 an extensive bacteriologic study was made of epidemic meningitis in one of the large military camps. The meningococcus carriers in the infected organizations were identified and isolated. For the cultures plain blood agar was found to be satisfactory and the material for culture was obtained from the nasopharynx by means of a simple uncovered wire swab. It was found that 3-6% of the men examined were meningococcus carriers. The majority of these carriers, however, were of the temporary type; only 1.2% of the total number of suspects examined proved to be chronic carriers. Chronic meningococcus carriers, as distinguished from the temporary type, often harbor great numbers of meningococci in the secretions of the nose and throat. The number of carriers was found to be high among those coming in contact with meningitis cases.

In a study of the biologic reactions of 150 strains of meningococci from different sources two large biologic groups were differentiated by means of macroscopic agglutination tests using monovalent serums. The agglutination reactions were in most instances definite and specific but a number of atypical and inagglutinable strains were met with in each group. The atypical strains however did not differ enough from the other members of the group to warrant different classification as

determined by agglutination. The classification of the inagglutinable strains was accomplished by means of agglutination with monovalent serums prepared from these strains; these serums yielded specific reactions with organisms of one or the other main type.

The biologic type of meningococcus predominant in the camp epidemic was identical with the type prevailing among the chronic carriers, but different from the type of meningococcus causing the majority of the sporadic cases of cerebrospinal fever in Chicago. Also the type of meningococcus found in the cases and in the corresponding immediate contacts was in every instance the same. These facts suggest that there is a close relationship between cases of epidemic meningitis and meningococcus carriers.

THERMOSTABILE AND THE SO-CALLED THERMO-LABILE HEMOLYSINS

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F. H. Thiele and Dennis Embleton[1] in a paper on the "Evolution of the Antibody" present many experiments to show that the so-called amboceptor is a development of the complement, the evolution being gradually traceable from the one to the other. They used both normal and acquired hemolytic antibodies, and noticed with both that serums which were markedly hemolytic in the fresh state, not only lost this property on inactivation, but that this power was not restored or only incompletely by the addition of complement.

In their experiments with normal hemolysins advantage was taken of the natural antibodies present in 5 rabbit and 2 human serums for sheep cells. Five of these serums could not be reactivated after heating at 55 C. for 30 minutes; the other two were reactivated to a certain extent. My experiments closely corroborate these results. In 6 of their fresh serums they were able to separate the amboceptor by absorption in the cold, but report one serum in which the amboceptor and complement were both absorbed together. In my experiments summarized in this article it will be evident that in no case was a real thermolabile hemolysin found, and that with every experiment with normally hemolytic serum, amboceptor could be absorbed from the fresh serum and also from the heated, nonactivatable serum. By "thermolabile" is meant destructibility when heated in a water-bath at 56 C. for 30 minutes.

TECHNIC

Hemolytic test tubes of 4 c c capacity were used. The required amounts of hemolytic serum, erythrocyte suspension, and complement when used, were placed in the test tubes and enough isotonic NaCl solution added to make 1 c c. After shaking, the tubes were placed in a 37 C. water-bath for 30 minutes, shaken again, and reincubated for 30 minutes. The results were then read and the tubes placed in the cold over night and any change from previous reading was noted. In the absorption experiments the tubes were cooled to between — 10 C. and 0 C. in a CaCl₂ powdered ice mixture; the tubes when centrifuged

were kept surrounded by a sufficient quantity of the cooling mixture. The exposed erythrocytes were washed with isotonic NaCl solution cooled to 0 C.

Inactivated serum was obtained by heating the fresh serum at 56 C. for 30 minutes in a water-bath. Complement was obtained from rabbits, guinea-pigs, swine, calves, man, and dogs, and titrated before using.

The erythrocytes were washed 3 times in isotonic NaCl solution and used in dose of 0.1 c c of a 10% suspension.

In all cases complement was tested for natural hemolysin, and when amboceptor was present, as in the dog serum, it was removed by absorption with the homologous erythrocyte. The salt solution was always tested for isotonicity.

The results are expressed the same as in the Wassermann reaction. A negative (—) sign means complete hemolysis; 1 + means nearly complete hemolysis; 2 + means 50% hemolysis; 3 + means slight hemolysis; 4 + means no hemolysis.

TABLE 1

Action of Dog Serum on Various Erythrocytes

Serum of Dog 1	Erythrocytes						
	Rabbit	G. Pig	Dog	Duck	Calf	Swine	Man
1. Fresh normal dog serum, 0.1 cc......	±	—	4+	—	—	—	1+
2. Inactivated dog serum, 0.3 cc, + rabbit complement, 1 unit.............	4+	4+	4+	4+	3+ ·	—	4+
3. Inactivated dog serum, 0.3 cc, + swine complement, 1 unit...........	4+	4+	4+	4+	3+	—	4+
4. Inactivated dog serum, 0.3 cc, + calf complement, 1 unit.............	4+	4+	4+	4+	4+	—	4+
5. Inactivated dog serum, 0.3 cc, + guinea-pig complement, 1 unit......	4+	4+	4+	4+	4+	—	4+
6. Inactivated dog serum, 0.3 cc, + human complement, 1 unit...........	4+	2+	4+	4+	4+	3+	4+
7. Inactivated dog serum after saturation, + complement, 1 unit........	4+	4+	4+	4+	4+	4+	4+
8. Exposed erythrocytes, + complement, 1 unit	—	—	4+	—	—	—	—

It will be observed from the results of Exper. 1 that Dog 1 had natural hemolysins for rabbit, guinea-pig, duck, calf, swine and human erythrocytes. By referring to Thiele and Embleton's work it will be seen that they absorbed the hemolysin from their fresh serum, but never attempted to absorb the hemolysins from their inactivated serum, evidently just taking it for granted that the amboceptor was destroyed from the fact that the power was not restored on the addition of fresh complement. The results shown on Lines 7 and 8 of Table 1 are of interest, as they demonstrate clearly that the hemolysin of Dog 1 serum when heated to 56 C. for 30 minutes is not affected deleteriously in the least; Line 7 shows that the inactivated serum loses all hemolysins on saturation with the respective erythrocytes; Line 8 shows that the erythrocytes (exposed) from Line 7 after saturation in the respective inactivated serum are completely laked on addition of proper complement. In this case complement was obtained by absorbing all hemol-

ysins from the fresh Dog 1 serum by excess of the respective erythrocytes and using the supernatant fluid.

The results of this experiment would seem to be in direct opposition to the argument of Thiele and Embleton for the evolution of the thermolabile complement through the so-called thermolabile antibodies to the thermostabile antibodies, as it has shown that the hemolytic antibodies studied in this paper are intrinsically thermostabile.

TABLE 2

ACTION OF CALF SERUM ON VARIOUS ERYTHROCYTES

Serum of Calf	Erythrocytes				
	Rabbit	G. Pig	Duck	Swine	Human
Fresh normal calf serum, 0.1 cc............................	—	—	±	++	—
Inactivated calf serum, 0.2 cc, + complement, 1 unit....	4+	4+	4+	4+	4+
Exposed erythrocytes, after saturation in inactivated serum, + complement, 1 unit........................	—	—	—	4+	—

Exper. 2 with calf blood serum merely substantiates the results of Exper. 1, namely, that the so-called thermolabile natural hemolysins are really thermostabile and can be absorbed in their original power from the inactivated serum.

TABLE 3

ACTION OF RABBIT SERUM ON VARIOUS ERYTHROCYTES

Serum of Rabbit	Erythrocytes				
	Rabbit	G. Pig	Duck	Swine	Human
Fresh normal rabbit serum, 0.1 cc........................	1+	—	±	4+	4+
Inactivated rabbit serum, 0.2 cc, + complement, 1 unit.	4+	4+	4+	4+	4+
Exposed erythrocytes, after saturation in inactivated rabbit serum, + complement........................	—	—	—	4+	4+

The results of Exper. 3 on rabbit serum are similar to those of Exper. 1 on Dog 1 serum and to Exper. 2 on Calf serum. Kolmer and Williams[2] sum up very thoroughly the obvious natural hemolysins in inactivated normal rabbit serum for goat, sheep, dog, human, hog, ox, chicken, guinea-pig and white rat erythrocytes; however, they fail to take into account those hemolysins which appear to become destroyed during the heating, and it would be very interesting to know what percentage of their inactivated serums, in which they report no hemolysins, would show hemolysins on saturation in the cold with erythrocytes. As all the hemolysins studied in this article are thermostabile,

[2] Jour. Infect. Dis., 1913, 13, p. 96.

it would be plainly a misnomer to classify part of them as thermolabile, and it is suggested, first, that all those hemolysins that can be titrated direct, including those which are still detectable after inactivation as well as those in fresh serums, be designated as "obvious" hemolysins; second, that those hemolysins that are not detectable by direct titration after inactivation be called "masked" hemolysins. These new terms will be used henceforth in this and following papers, for the sake of brevity and clearness.

I have in preparation a paper on the effect of inanition and special diets on hemolysins and an attempt will be made therein to throw some light on this curious phenomena of the obvious hemolysins being converted to the masked hemolysins by heating at 56 C. for 30 minutes, the hemolytic power remaining undiminished.

TABLE 4

Action of Swine Serum on Various Erythocytes

Serum of Swine	Erythrocytes						
	Rabbit	G. Pig	Dog	Duck	Calf	Swine	Human
Fresh normal swine serum, 0.15 cc.......	4+	—	4+	—	4+	4+	4+
Inactivated serum, 0.3 cc, + complement	4+	4+	4+	4+	4+	4+	4+
Exposed erythrocytes, after saturation in inactivated serum, + complement	4+	—	4+	—	4+	4+	4+

Exper. 4 with swine blood serum gives similar results to those of the three previous experiments. In all cases it will be seen that the masked hemolysins are evidently as strong as the obvious hemolysins.

TABLE 5

Action of Sheep Serum on Various Erythrocytes

Serum of Sheep	Erythrocytes			
	Dog	G. Pig	Rabbit	Human
Fresh normal sheep serum, 0.1 cc...............................	4+	—	—	—
Fresh normal sheep serum, 0.1 cc. + guinea-pig complement, 1 unit	—	—	—	—
Inactivated sheep serum, 0.3 cc, + complement, 1 unit.........	4+	—	4+	4+
Exposed erythrocytes, after saturation in inactivated serum, + complement, 1 unit.............................	—	—	—	—

The results of Exper. 5 resemble those of the preceding four experiments. In Line 2, however, it will be noticed that the fresh sheep serum is complemented with guinea-pig serum for dog erythrocytes, but is not complemented with its own (sheep) serum. It will

be well to state here that the results of the addition of complement to
a fresh serum are given only when these results are different from
those when the fresh serum is used alone. H. Windsor Wade[3] gives
two tables of statistical data concerning the relative efficiency of the
Hecht-Weinberg (antisheep) and the Tschernogubow (antiguinea-pig)
modifications of the Wassermann reaction; he reports on 1,000 cases
(consecutive specimens obtained in routine hospital practice) and finds
that of the 500 examined with the antiguinea-pig cell system only 21,
or 4.2%, did not give one unit of hemolysis, and of the 500 examined
with the antisheep cell system 77, or 15.4%, did not give one unit of
hemolysis. In this work of Wade's no foreign complement was used;
it would be very interesting to know how many of his negative cases
would have caused lysis on the addition of foreign complement. Fresh
serum was used in all of his experiments, hence his results show the
obvious hemolysins present, but none of the masked hemolysins.

TABLE 6

ACTION OF HUMAN SERUM ON VARIOUS ERYTHROCYTES

Human Serum	Erythrocytes			
	Dog	G. Pig	Rabbit	Sheep
Fresh normal human serum, 0.1 cc..................................	—	—	—	—
Inactivated human serum, 0.2 cc, + complement, 1 unit........	—	4+	4+	2+
Exposed erythrocytes, after saturaton in inactivated serum, + complement, 1 unit...	—	—	—	—

Kolmer and Casselman[4] report the results of a large number of
experiments with the natural hemolysins of inactivated human serum
on sheep, dog, ox, goat, hog, rat, chicken, horse, rabbit and guinea-pig
erythrocytes. They found that 93% of the inactivated human serums
contained natural hemolysins for sheep erythrocytes; 82% for dog;
50% for ox; 24% for goat; 12% for rat; 8% for chicken; 4% for
horse and rabbit, and only 2% for guinea-pig. They concluded that the
swine, rat, chicken, horse, rabbit or guinea-pig systems were preferable
to the sheep or dog systems for complement fixation with human
serum. The results in Line 2 of my Exper. 6 on human serum check
very well with the work of Kolmer and Casselman; Line 3 shows that
the hemolytic power is not destroyed by the inactivation, but has simply
become masked. Note that Wade finds that 95.8% of fresh human

[3] Jour. Med. Research, 1916, 34, p. 113.
[4] Jour. Infect. Dis., 1915, 16, p. 441.

serum contain hemolysins for guinea-pig erythrocytes while Kolmer and Casselman show that only 2% of inactivated human serums contain the guinea-pig hemolysin; the difference between these percentages, or 93.8%, represents the amount of obvious hemolysins which become masked on inactivating.

Thiele and Embleton, in addition to their work with natural hemolysins, injected seven rabbits with either human or sheep erythrocytes. They found that during the first 2 or 5 days the antibody formed was inactivated by heating; then later a part of it became thermostabile and finally most of it withstood heating. They considered these results as further evidence of the "evolution of the antibody" from complement to amboceptor. To check these results I inoculated 13 rabbits with human erythrocytes; after 3 days these animals were bled and their serums tested. In 3 of these serums no hemolysins could be demonstrated; in 5 others obvious hemolysins were found, but all became masked on inactivation, which latter, however, were absorbed in the same strength as found previous to inactivation; in one other serum only part of the obvious hemolysins became masked on inactivation and the titer of the combined obvious and masked hemolysins of the inactivated serum was the same as the obvious hemolysins of the fresh serum; in the remaining 4 serums the obvious hemolysins all became masked on inactivation, but in two of these the masked titrated somewhat higher than the obvious hemolysins of the fresh serums, while in the other two the titers of the masked were slightly lower than those of the obvious hemolysins. The results of this experiment on 13 rabbits agree essentially with Thiele and Embleton's results; but my experiments show that the hemolysin is not destroyed by inactivation and demonstrate clearly that the amboceptor can be absorbed in its original power, or even stronger, from the inactivated serum. Hence, their conclusions seem to be based on a fallacy and fail to coincide with the results published here.

CONCLUSIONS

As all of the hemolysins investigated are thermostabile it is a misnomer to term part of them thermolabile.

In view of the foregoing fact it is suggested that all of those hemolysins which can be titrated direct be called "obvious" hemolysins.

It is also suggested further that the term "masked" be applied to those "obvious" hemolysins which are not detectable by direct titration after inactivation.

As there are no thermolabile hemolysins, there can be no "evolution of the antibody" from a thermolabile complement through a thermo-labile hemolysin to a thermostabile hemolysin.

The immune hemolysin first formed on injection of the antigen is thermostabile.

THE ATTENUATION OF HOG CHOLERA VIRUS AND ITS EFFECT ON NORMAL HOGS

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The discovery by Dorset, Bolton and McBryde[1] that hog cholera is caused by a filtrable virus, marks the beginning of a new era in the investigation of this disease. Following this, the discovery of the Dorset-Niles method of preparing an antiserum for the prevention of hog cholera was another important step in advance. Recently investigators have devoted their studies to methods whereby the filtrable virus may be attenuated and used as a vaccine. In each case, as the review of the literature shows, the results have been much the same, namely, a failure to attenuate the virus of hog cholera with any degree of accuracy or success.

HISTORICAL

Boxmeyer, McClintock and Seffer[2] found that drying hog cholera virus at 37 C. or adding one-third its weight in glycerol did not attenuate the virus. Graham[3] states that virus heated at 60 C. for one hour did not produce sufficient immunity to protect hogs from cholera. He notes, also, that different strains of virus vary greatly. King[4] reports that attempts to attenuate hog cholera virus by various physical and chemical means, so that it could be used as a vaccine, were not successful. Dorset and Niles[5] were unsuccessful in their attempts to prepare a vaccine through an attenuated virus. Craig[6] found that virus heated in a water bath at 51-60 C. did not confer any immunity and later work[7] by the same author showed that he was not able to produce a successful vaccine. Lewis and others[8] report some success in the attenuation of hog cholera virus by injecting the virus into a hyperimmune hog and allowing it to remain for varying lengths of time. Healy and Gott[9] thought that possibly they had succeeded in attenuating hog cholera virus by mixing it with antiserum and incubating.

[1] Bureau Animal Industry, Bull. 180.
[2] Jour. Infect. Dis., 1905, 2, p. 351.
[3] Am. Vet. Rev., 41, p. 330.
[4] Kans. Exp. Sta. Bull., 157, pp. 37-90.
[5] U. S. Live Stock Sanitary Proceedings, 1911.
[6] Ind. Sta. Report, 1912, p. 79.
[7] Ibid., 1913, p. 76.
[8] Okla. Sta. Bull., 104, 1914.
[9] Jour. Infect. Dis., 1916, 19, p. 569.

EXPERIMENTAL WORK

In investigating the virus of hog cholera many obstacles are constantly met with, for as yet many of the phenomena connected with the disease are poorly understood. In most cases the disease is so closely associated with secondary invaders that they appear to be almost a part of the true infection, and their etiologic significance is still more or less a matter of inquiry. The lack of a specific test for hog cholera greatly handicaps the investigator and renders it more difficult to say just what does constitute a true cholera. The lack of a standard unit for measuring the virulence of hog cholera virus is another serious handicap. Reichel[10] and later Robbins[11] have both devised means for maintaining a virulent strain of hog cholera virus over considerable periods of time, but as yet no definite unit has been established for measuring the virulence of the virus. Until some method is devised whereby the virus can be standardized, investigators must work to a large extent in the dark. Aside from the difficulties mentioned, there is the great variation by different hogs in their resistance to the disease and the great variation in virulence of different strains of virus.

The results given in this article are taken from a series of experiments on the attenuation of hog cholera virus by different methods, and on the effect of this treated virus when injected into healthy hogs, which had not been rendered immune to cholera by exposure or vaccination.

' The experimental work differs from that of other investigators in the methods used for attenuation, the temperature and the quantity of heated material in each injection.

The virus was secured from different sources as will be noted in the different experiments. The work conducted by Dinwiddie[12] indicated that the serum-free corpuscles from hog cholera blood are more virulent than the plasma without the corpuscles from the same blood. In other words, that the causative organism is intracorpuscular. For this reason the blood used in these experiments was divided into two portions so that comparison could be made between the defibrinated blood and the blood corpuscles centrifuged as free as possible from serum. When the virus hog was killed one portion of the blood was collected in a sterile vessel, defibrinated and placed in tubes for heating. The other portion of the blood was collected in sterile salt solution 0.9%, filtered through gauze and the corpuscles washed as free as possible from serum by centrifuging nine times with sterile salt solution. In each case the quantity

[10] Jour. Infect. Dis., 1913, 12, p. 39.
[11] Ibid., 1916, 19, p. 708.
[12] Ark. Exp. Sta. Bull., No. 111, 1912.

of virus required for each injection was placed in the bottom of tubes by means of a sterile pipet, care being taken that none of the virus touched the tube near the mouth. The heating was then conducted in a water bath, each tube being stoppered with cotton and immersed in the water to a much greater depth than the depth of the virus so that there would not be any chance for any of the virus to remain unheated.

The hogs used for testing the material were secured from the surrounding country. In no case were they taken from farms where cholera had been prevalent or where vaccination had been practiced. The hogs varied in weight from 40-60 lbs. and were of various breeds. After inoculation each hog was placed in a small shed with wood floor, these sheds being inclosed in larger pens. Each shed was carefully disinfected twice, one week apart, before using.

Exper. 1.—The attenuation of hog cholera virus and serum-free virus cells by heating at 55 C. for 2 hours.

The strain of virus in this experiment came from the Kentucky State Serum Plant and had been passed through two generations of hogs. The blood was collected from a virus hog which had been inoculated 6 days previously with 5 c c of this virus and was at the height of infection when killed.

In each part of the experiment 5 pigs, weighing from 40-60 lbs. were used.

Each animal was given 5 c c of the heated material, except the 2 check pigs which were each given 5 c c of the unheated material. Separate sterile syringes were used for each injection and the material was injected into the muscles of the ham. Ten days after the first injection each pig, except the check pigs, was given a second injection consisting of 10 c c of the heated material, the same strain of virus being used as in the first injection. Each pig was kept in a separate shed and all precautions taken against outside infection.

TABLE 1

INJECTION OF VIRUS HEATED AT 55 C. FOR TWO HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Cholera
50	5 c c of virus heated at 55 C. for 2 hours	10 c c of virus heated at 55 C. for 2 hours. Temperature of pig when injected 105.2 F.	January 21
51	5 c c of virus heated at 55 C. for 2 hours	10 c c of virus heated at 55 C. for 2 hours. Temperature of pig when injected 105 F.	January 28
52	5 c c of virus heated at 55 C. for 2 hours	10 c c of virus heated at 55 C. for 2 hours. Temperature of pig when injected 105.8 F.	January 23
53	5 c c of virus heated at 55 C. for 2 hours	10 c c of virus heated at 55 C. for 2 hours. Temperature of pig when injected 106 F.	January 20
54	5 c c of above virus unheated	....	January 17

We observe that both the virus and serum-free virus cells were quite virulent by the fact that check Pig 54 survived 9 days and 54E 7 days. The postmortem conditions were nearly alike, being that of acute cholera in both cases. None of the animals recovered from the disease; 21 days being the greatest length of time that any pig

survived. Pig 50A and the 2 check pigs did not live to receive the second injection. It was evident that heating the material attenuated it to a slight extent, as both of the check pigs succumbed several days earlier than the remaining pigs in the experiment. There was but little difference in the virulence of the heated material used in the two parts of the experiment as is shown in Tables 1 and 2. The serum-free corpuscles may have been slightly more virulent than the virus.

TABLE 2

INJECTION OF CORPUSCLES HEATED AT 55 C. FOR TWO HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Cholera
50A	5 c c of serum free corpuscles heated at 55 C. for 2 hours	....	January 18
51B	5 c c of serum free corpuscles heated at 55 C. for 2 hours	10 c c of serum-free corpuscles heated at 55 C. for 2 hours. Temperature of pig when injected 107.1 F.	January 20
52C	5 c c of serum free corpuscles heated at 55 C. for 2 hours	10 c c of serum-free corpuscles heated at 55 C. for 2 hours. Temperature of pig when injected 104.9 F.	January 29 chronic cholera
53D	5 c c of serum free corpuscles heated at 55 C. for 2 hours	10 c c of serum-free corpuscles heated at 55 C. for 2 hours. Temperature of pig when injected 105.3 F.	January 21
54E	5 c c of above cells unheated	....	January 15

TABLE 3

INJECTION OF VIRUS HEATED AT 55 C. FOR FOUR HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Cholera
55	5 c c of hog cholera virus heated at 55 C. for 4 hours	10 c c of virus heated at 55 C. for 4 hours. Temperature of pig when injected, 104 F.	January 27
56	5 c c of hog cholera virus heated at 55 C. for 4 hours	10 c c of virus heated at 55 C. for 4 hours. Temperature of pig when injected, 105.8 F.	January 23
57	5 c c of hog cholera virus heated at 55 C. for 4 hours	10 c c of virus heated at 55 C. for 4 hours. Temperature of pig when injected, 105.8 F.	February 2
58	5 c c of hog cholera virus heated at 55 C. for 4 hours	10 c c of virus heated at 55 C. for 4 hours. Temperature of pig when injected, 105.2 F.	January 24
59	5 c c of above virus unheated	....	January 16

Exper. 2.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 55 C. for 4 hours.

The strain of virus used in this experiment was the same as that in Exper. 1 and had been passed through two generations since receipt. The blood was

collected from a virus pig which had been inoculated 8 days previously with 5 cc of this virus and was in the height of infection when killed.

Five pigs, weighing from 40-60 lbs. were used in each part of the experiment.

The same methods were followed and quantities used for inoculation as in Exper. 1.

TABLE 4

INJECTION OF CORPUSCLES HEATED AT 55 C. FOR FOUR HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Cholera
55A	5 cc of serum-free corpuscles heated at 55 C. for 4 hours	10 cc of serum-free corpuscles heated at 55 C. for 4 hours. Temperature of pig when injected, 106.4 F.	January 27
56B	5 cc of serum-free corpuscles heated at 55 C. for 4 hours	....	January 18
57C	5 cc of serum-free corpuscles heated at 55 C. for 4 hours	10 cc of serum-free corpuscles heated at 55 C. for 4 hours. Temperature of pig when injected, 105 F.	January 23
58D	5 cc of serum-free corpuscles heated at 55 C. for 4 hours	....	January 18
59E	5 cc of above corpuscles unheated	....	January 19

The material used in both parts of this experiment was virulent, as shown by the length of time that the check pigs survived, Pig. 59 living 8 days and 59E 11 days. The postmortem findings were those of acute cholera. None of the pigs recovered from the disease, 25 days being the greatest length of time that any pig survived.

In the second part of the experiment (Table 4) only 2 of the pigs lived to receive the second injection. Here again as in Exper. 1, heating seemed to attenuate the material, as the check pigs which received the unheated material, succumbed much earlier than the remaining pigs of the experiment. The serum-free corpuscles were possibly more virulent than the other material, but there was but little difference. The cases which lingered longest began to show slight symptoms of chronic cholera. The results indicate that there was but little difference in the virulence of the material when heated 4 hours and that heated 2 hours.

Exper. 3.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 60 C. for 4 hours.

The strain of virus used in this experiment was secured from the Arkansas State Serum Plant, 5 cc being injected into a pig weighing approximately 50 lbs. At the end of 8 days the pig was killed while in the height of infection and the blood collected for the experiment.

Five pigs weighing from 50-60 lbs. were used in each part of the experiment.

The same methods were followed and same quantities of material used for the inoculations as in Exper. 1. In this experiment the heated blood and serum-free corpuscles both coagulated. In order to make the injections it was necessary to add sufficient sterile salt solution to the coagulated material to place it in suspension.

TABLE 5

INJECTION OF VIRUS HEATED TO 60 C. FOR FOUR HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Chole
60	5 c c of hog cholera virus heated at 60 C. for 4 hours	10 c c of virus heated at 60 C. for 4 hours. Temperature of pig when injected, 102.4 F.	February 24
61	5 c c of hog cholera virus heated at 60 C. for 4 hours	10 c c of virus heated at 60 C. for 4 hours. Temperature of pig when injected, 102 F.	February 26
62	5 c c of hog cholera virus heated at 60 C. for 4 hours	10 c c of virus heated at 60 C. for 4 hours. Temperature of pig when injected, 102.6 F.	February 7
63	5 c c of hog cholera virus heated at 60 C. for 4 hours	10 c c of virus heated at 60 C. for 4 hours. Temperature of pig when injected, 103.7 F.	January 21
64	5 c c of above virus unheated	....	January 17

TABLE 6

INJECTION OF CORPUSCLES HEATED TO 60 C. FOR FOUR HOURS

Pig	Material, First Injection January 8	Material, Second Injection, January 18	Animal Died from Cholera
60A	5 c c of serum-free corpuscles heated at 60 C. for 4 hours	10 c c of serum-free corpuscles heated at 60 C. for 4 hours. Temperature of pig when injected, 102.8 F.	Feb. 20; chronic cholera
61B	5 c c of serum-free corpuscles heated at 60 C. for 4 hours	10 c c of serum-free corpuscles heated at 60 C. for 4 hours. Temperature of pig when injected, 103.3 F.	February 3
62C	5 c c of serum-free corpuscles heated at 60 C. for 4 hours	10 c c of serum-free corpuscles heated at 60 C. for 4 hours. Temperature of pig when injected, 101.8 F.	Feb. 27; chronic cholera
63D	5 c c of serum-free corpuscles heated at 60 C. for 4 hours	10 c c of serum-free corpuscles heated at 60 C. for 4 hours. Temperature of pig when injected, 103 F.	February 24 chronic cholera
64E	5 c c of above cells unheated	....	January 15

The virus and serum-free corpuscles were both quite virulent as shown by the length of time the 2 check pigs survived, Pig 64 living 9 days and 64E 7 days. Six of the remaining pigs survived a month or longer, and in each case the lesions found on necropsy varied from subacute to those of chronic cholera. The 2 remaining pigs lived 13 days and 26 days, respectively, and both showed marked lesions of hog cholera.

It was evident that heating the material attenuated it to some extent, as all animals except the check pigs lived to receive the second injection. The temperature of all pigs receiving the second injection was practically normal when the second injection was given, but outward symptoms would indicate that the animals had not entirely recovered from the first injection. There did not seem to be any difference in the virulence of the virus and serum-free virus cells after heating.

Exper. 4.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 65 C. for 2 hours.

The strain of virus used in this experiment was secured from the Arkansas State Serum Plant and had been passed through two generations of pigs since coming to the laboratory. The blood for this experiment was collected from a virus pig which had been inoculated 6 days previously with 5 c c of the virus and was in the height of infection when killed.

As in the previous experiments, 5 pigs weighing from 40-60 lbs. were used in each part of the experiment.

The same methods were followed and same quantities of material used for the inoculations as in Exper. 1. The coagulated material was placed in suspension in the same manner as in Exper. 3.

TABLE 7

INJECTION OF VIRUS HEATED AT 65 C. FOR TWO HOURS

Pig	Material, First Injection March 25	Material, Second Injection, April 4	Results
70	5 c c of hog cholera virus heated at 65 C. for 2 hours	10 c c of virus heated at 65 C. for 2 hours. Temperature of pig when injected, 101 F.	Died April 25; chronic cholera
71	5 c c of hog cholera virus heated at 65 C. for 2 hours	10 c c of virus heated at 65 C. for 2 hours. Temperature of pig when injected, 102 F.	Lived; given 5 c c of virus April 5
72	5 c c of hog cholera virus heated at 65 C. for 2 hours	10 c c of virus heated at 65 C. for 2 hours. Temperature of pig when injected, 105.2 F.	Died April 27
73	5 c c of hog cholera virus heated at 65 C. for 2 hours	10 c c of virus heated at 65 C. for 2 hours. Temperature of pig when injected, 101.8 F.	Died April 24
74	5 c c of above virus unheated	....	Died April 2

The virus and serum-free corpuscles were both quite virulent as was indicated by the length of time which the 2 check pigs survived, Pig 74 living 8 days and 74E 11 days. One pig survived all injections, although it was in a badly stunted condition when the experiment was closed. Three of the pigs lived a month or slightly longer, and all 3 showed marked lesions of chronic hog cholera on necropsy. One animal only lived 10 days and showed lesions of acute hog cholera.

The 2 remaining animals showed lesions of a subacute disease. All the pigs, except one, showed a normal temperature when the second injection was given.

There was but little difference in the virulence of this material, which was heated at 65 C. for 2 hours, and that in the previous experiment which was heated at 60 C. for 4 hours.

There did not seem to be any difference in the virulence of the virus and the serum-free corpuscles after heating.

TABLE 8

INJECTION OF CORPUSCLES HEATED AT 65 C. FOR TWO HOURS

Pig	Material, First Injection March 25	Material, Second Injection, April 4	Animal Died
70A	5 c c of serum-free corpuscles heated at 65 C. for 2 hours	10 c c of serum-free corpuscles heated at 65 C. for 2 hours. Temperature of pig when injected, 103 F.	April 30 chronic cholera
71B	5 c c of serum-free corpuscles heated at 65 C. for 2 hours	10 c c of serum-free corpuscles heated at 65 C. for 2 hours. Temperature of pig when injected, 102.5 F.	April 20 chronic cholera
72C	5 c c of serum-free corpuscles heated at 65 C. for 2 hours	10 c c of serum-free corpuscles heated at 65 C. for 2 hours. Temperature of pig when injected, 102.1 F.	April 18 chronic cholera
73D	5 c c of serum-free corpuscles heated at 65 C. for 2 hours	....	April 4
74E	5 c c of above serum-free corpuscles unheated	....	April 5

Exper. 5.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 70 C. for 2 hours.

This strain of virus was secured from the Michigan State Serum Plant and was used as soon as it reached the laboratory, 5 c c being injected into a pig of approximately 50 lbs. weight. The animal was killed on the 8th day and the blood collected for the experiment.

Five pigs weighing 50-60 lbs. were used in each part of the experiment.

The same methods were followed and the same quantities used for the inoculations as in Exper. 1. The coagulated material was placed in suspension in the same manner as in Exper. 3. After a month had passed 6 of the animals were in fair condition and each was given an injection of 5 c c of virulent virus to determine whether or not any immunity had been built up by the 2 previous injections.

The virus and serum-free corpuscles were both virulent as shown by the length of time that the 2 check pigs lived, Pig 84 surviving 9 days and 84E 10 days. The heated material showed much more evidence of attenuation than in previous experiments. While only one pig recovered, nearly all survived longer than in any of the previous

experiments. Three of the animals lived 2 months or longer. Six of
the animals lived one month or longer and received a third injection,
but only one pig had enough immunity to withstand the third injection.
Pig 80A lived 20 days and showed only lesions of acute cholera on
necropsy. All of the other animals, except the check pigs, showed
marked lesions of chronic cholera.

TABLE 9

INJECTION OF VIRUS HEATED AT 70 C. FOR TWO HOURS

Pig	Material, First Injection March 25	Material, Second Injection, April 4	Results
80	5 c c of hog cholera virus heated at 70 C. for 2 hours	10 c c of hog cholera virus heated at 70 C. for 2 hours. Temperature of pig when injected 102 F.	Died, May 14; chronic cholera
81	5 c c of hog cholera virus heated at 70 C. for 2 hours	10 c c of hog cholera virus heated at 70 C. for 2 hours. Temperature of pig when injected 101.5 F.	Given 5 c c of strong virus on April 25. Died June 1; chronic cholera lesions on necropsy.
82	5 c c of hog cholera virus heated at 70 C. for 2 hours	10 c c of hog cholera virus heated at 70 C. for 2 hours. Temperature of pig when injected 102.5 F.	Given 5 c c of strong virus on April 25. Died May 20; chronic cholera lesions on necropsy
83	5 c c of hog cholera virus heated at 70 C. for 2 hours	10 c c of hog cholera virus heated at 70 C. for 2 hours. Temperature of pig when injected 102 F.	Given 5 c c of strong virus on April 25; lived
84	5 c c of above virus unheated	....	Died, April 3; acute cholera

TABLE 10

INJECTION TO CORPUSCLES HEATED AT 70 C. FOR TWO HOURS

Pig	Material, First Injection March 25	Material, Second Injection, April 4	Results
80A	5 c c of serum-free corpuscles heated at 70 C. for 2 hours	10 c c of serum-free corpuscles heated at 70 C. for 2 hours. Temperature of pig when injected 103 F.	Died April 14; acute cholera
81B	5 c c of serum-free corpuscles heated at 70 C. for 2 hours	10 c c of serum-free corpuscles heated at 70 C. for 2 hours. Temperature of pig when injected 102.2 F.	Given 5 c c of strong virus on April 25. Died May 3; chronic cholera
82C	5 c c of serum-free corpuscles heated at 70 C. for 2 hours	10 c c of serum-free corpuscles heated at 70 C. for 2 hours. Temperature of pig when injected 102.6 F.	5 c c strong virus April 25. Died, May 27; chronic cholera
83D	5 c c of serum-free corpuscles heated at 70 C. for 2 hours	10 c c of serum-free corpuscles heated at 70 C. for 2 hours. Temperature of pig when injected 101.8 F.	5 c c strong virus April 25. Died June 4; chronic cholera
84E	5 c c of above serum-free corpuscles unheated	....	Died April 4; acute cholera

From the length of time that the different animals survived it would appear that the material was attenuated by heating and some immunity built up by the injections, but not enough to withstand an injection of strong unheated virus.

Exper. 6.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 70 C. for 4 hours.

This was also a Michigan strain of virus and had been passed through four generations of pigs since coming to the laboratory. The blood used in this experiment was collected from a pig which had been inoculated 7 days previously with 5 c c of this virus and was in the height of infection when killed.

Five pigs averaging 40-60 lbs. were used in each part of the experiment.

This experiment differs considerably from the previous experiments, only ⅒ of the quantity or 0.5 c c of the heated material being used for the inoculations. The check pigs each received 0.5 c c of the unheated material. At the end of 21 days each pig was given 1 c c strong unheated virus to determine whether or not any immunity had been built up by the first injection.

TABLE 11

INJECTION OF VIRUS HEATED AT 70 C. FOR FOUR HOURS

Pig	Material, First Injection October 2	Material, Second Injection, October 23	Results
90	0.5 c c of hog cholera virus heated at 70 C. for 4 hours	1 c c of unheated virus	Recovered
91	0.5 c c of hog cholera virus heated at 70 C. for 4 hours	1 c c of unheated virus	Recovered
92	0.5 c c of hog cholera virus heated at 70 C. for 4 hours	1 c c of unheated virus	Recovered
93	0.5 c c of hog cholera virus heated at 70 C. for 4 hours	....	Too sick for second injection; died November 20; cholera lesions on necropsy
94	0.5 c c of virus, unheated	....	Died October 10; acute cholera

The length of time which the check pigs survived would indicate that the unheated material was fully virulent, check Pig 94 living 8 days and 94E 10 days after inoculation. The heated material showed a much greater attenuation than in any of the previous experiments. Five of the pigs withstood an injection of virulent hog cholera virus 21 days after the first injection of heated material, and finally recovered. They were badly stunted, however. The 3 remaining pigs lived a month or longer, but finally all developed chronic cholera. There was evidently some immunity produced by the first injection, but not enough to protect all of the animals from 1 c c of virulent virus.

TABLE 12

INJECTION OF CORPUSCLES HEATED AT 70 C. FOR FOUR HOURS

Pig	Material, First Injection October 2	Material, Second Injection, October 23	Results
90A	0.5 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died, Nov. 3; chronic cholera
91B	0.5 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Recovered
92C	0.5 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died, Nov. 17; chronic cholera
93D	0.5 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Recovered
94E	0.5 c c of serum-free corpuscles unheated	....	Died, October 12; acute cholera

Exper. 7.—The attenuation of hog cholera virus and serum-free corpuscles by heating at 70 C. for 4 hours.

The blood used in this experiment was the same as that used in the preceding experiment and was secured from the same virus pig.

Five pigs weighing from 40-50 lbs. were used in each part of the experiment.

Each animal was given 0.25 c c of the heated material, except the check pigs which were given 0.25 c c of the unheated material. After 21 days a second injection, consisting of 1 c c of virulent unheated virus, was given each animal except the check pigs. This virus was the same strain as that used in the first injection.

TABLE 13

INJECTION OF 0.25 C C OF VIRUS HEATED AT 70 C. FOR FOUR HOURS

Pig	Material, First Injection October 2	Material, Second Injection, October 23	Results
95	0.25 c c of virus heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Recovered
96	0.25 c c of virus heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Recovered
97	0.25 c c of virus heated at 70 C. for 4 hours	....	Died October 11; acute cholera
98	0.25 c c of virus heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died Nov. 8; chronic cholera
99	0.25 c c of above virus unheated	....	Died October 12; acute cholera

The material of this experiment was virulent, check Pig 99 surviving 10 days and 99E 7 days. Four of the pigs recovered from the inoculations, but one animal died in 9 days with acute cholera. The 3 remaining animals survived for a month or longer, finally developing cases of chronic cholera and had well marked chronic lesions. There did not seem to be any great difference in the results with 0.5 c c of

heated material and with 0.25 c c. There was a marked difference, however, in the results of these two experiments and the preceding ones in which larger quantities of heated material were used.

TABLE 14

INJECTION OF 0.25 C C CORPUSCLES HEATED AT 70 C. FOR FOUR HOURS

Pig	Material, First Injection October 2	Material, Second Injection, October 23	Results
95A	0.25 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died Nov. 26; chronic cholera
96B	0.25 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died Nov. 11; chronic cholera
97C	0.25 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Recovered
98D	0.25 c c of serum-free corpuscles heated at 70 C. for 4 hours	1 c c of virulent unheated virus	Died Nov. 17; chronic cholera
99E	0.25 c c of serum-free corpuscles unheated	....	Died October 9; acute cholera

SUMMARY

The virus used in each of these experiments was fully virulent, until heated, as shown by the length of time (6-10 days) required to kill the check pigs. Heating at 55 C. for 2 hours attenuated the material to a slight extent as did also heating at 55 C. for 4 hours, at 60 C. for 4 hours, and at 65 C. for 2 hours. There was but little difference in effect on normal pigs of the material heated at these temperatures, the material in practically every case being virulent enough to cause hog cholera.

Material heated at 70 C. for 2 hours showed considerable evidence of attenuation when injected in quantities of 5 c c. When heated at 70 C. for 4 hours in quantities of 0.5 and 0.25 c c, greater evidence of attenuation was shown than in any of the other experiments, and in only one case, Pig. 97, was the material virulent enough to cause disease. In the remaining animals some immunity was produced from the first injection, although all did not withstand an injection of 1 c c of virulent unheated hog cholera virus 21 days later. Although a number of the animals recovered in these last two experiments they were in a badly stunted condition. In all experiments the animals inoculated with heated material soon developed a marked cachexia and finally died in an emaciated condition, in most cases showing typical

lesions of chronic hog cholera. Some pigs were more resistant to the heated material than others.

There was but little, if any, difference in the virulence of the virus and the serum-free corpuscles.

The results of these experiments would indicate that hog cholera virus can be attenuated to a certain extent by heating, but this heated material is not suitable as vaccine, because in many cases it is apt to cause the disease.

CULTURES OF A LEPTOTHRIX FROM A CASE OF PARINAUD'S CONJUNCTIVITIS

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This form of chronic conjunctivitis is characterized by the unilateral development of nodules beneath the conjunctiva, with involvement of the corresponding preauricular gland. Its etiology has been obscure since the first description by Parinaud in 1889. We have not reviewed all the available literature. However, Karl Hoor[1] has reviewed 43 cases and reported one of his own; and F. H. Verhoeff and G. S. Derby[2] have reviewed 22 cases in addition to one of their own.

In many cases the inoculations of guinea-pigs and rabbits have proved the absence of tubercle bacilli. In the cases in which tubercle bacilli have been demonstrated it seems probable that they either concurrently or secondarily accompanied the true etiologic agent.

In 1913, Verhoeff[3] made a notable contribution. In 11 of 12 consecutive cases examined histologically he found, in addition to the same characteristic histologic picture, a minute filamentous organism. The filaments were present in irregular masses, from 10-60 μ in diameter, near or within the areas of cell necrosis which are especially prominent just beneath the epithelium. They can also be found in the superficial lymph spaces and this, according to Verhoeff, explains the early involvement of the regional glands.

The individual filaments were extremely delicate, about the diameter of the influenza bacillus; and varied from a few microns to 30 microns in length. They were straight, or more often irregularly curved or bent. They did not stain readily and were best demonstrated by a modified Gram's stain when the filaments exhibited rather regularly distributed granules. These granules varied in size and were "never exactly centered in the axis of the filament but project noticeably above its surface." No branching of the filaments was observed. Verhoeff suggested that it might be provisionally classed as a leptothrix.

[1] Monatsbl. f. Augenh., 1906, 44, p. 289.
[2] Arch. Ophth., 1904, 33, p. 389.
[3] Arch. Ophth., 1913, 42, p. 345.

We believe we have cultivated this leptothrix but do not insist on its identity with that described by Verhoeff as our organism does not exhibit the granular form.

REPORT OF CASE

History.—H. H., white, boy, aged 14 years, was admitted to the Cincinnati General Hospital, April 4, 1917; discharged cured, May 21, 1917. Did not feel well for a period of ten days before admission. The right eye began to inflame together with a swelling of the preauricular glands on the same side. There was no history of having played with any domestic animals nor had there been any illness in the family. On admission the eye showed decided ptosis with evidence of considerable conjunctivitis. There were no symptoms involving the cornea or iris. On everting the lids large nodular masses were found in the upper and lower lids. The preauricular glands were enlarged forming a mass about 1½ by 1 inch and elevated about ½ inch above the surrounding tissues (Fig. 1). During 25 days after admission the patient had an irregular fever ranging from 97-100 F. The treatment of the lids consisted in daily applications of 1% silver nitrate and the instillation of a 25% argyrol solution.
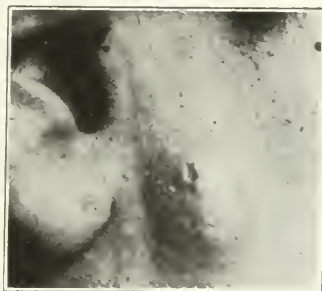
Fig. 1.—The preauricular gland some time after it had been opened.

Bacteriologic Examination.—Smears and cultures made at the time of admission from the mucopurulent secretion on the conjunctiva yielded nothing excepting 2 colonies of a diphtheroid bacillus. A 250 gm. guinea-pig inoculated intraperitoneally with the same material remained well during 20 days. When killed it was found to be normal.

Smears and cultures were made from pus collected from the preauricular gland 8, 10, and 17 days after admission. The smears were fixed by heat and by methyl alcohol and were stained with Loeffler's methylene blue, Bordet's carbol toluidin blue, Nicolle's carbol gentian violet, anilin Hoffman's violet, the tubercle method, and Giemsa without revealing any micro-organisms.*

The cultures were made on + 0.5 agar slants containing dextrose or maltose, with and without human blood; glycerol agar with rabbit blood; .oiled broth containing ascites fluid and sterile guinea-pig kidney, and Dorset's egg medium.

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* Prolonged staining in dilute warm Giemsa's stain was not used. This method has twice revealed bacteria which were not found by any of the ordinary methods of staining—once enormous numbers of filaments containing metachromatic granules and resembling B. fusiformis, apparently in pure culture in an abscess of the pancreas; and again a delicate filamentous organism in the pus of a case of hand infection and in the pus from the secondary meningitis which developed in this case.

They were incubated under aerobic, partial tension and anaerobic conditions at 37 C. and watched for about a month before being discarded as sterile. Following the isolation of the leptothrix, described below from Mouse 1, on coagulated egg yolk, pus collected from the preauricular gland 17 days after the patient was admitted was planted on this medium and incubated under anaerobic and partial tension conditions. These cultures were not examined for 2 weeks when the partial tension slants showed some very fine colonies of minute polymorphous rods resembling those found in the abscesses in Mouse 1. Only once was another organism encountered in the cultures and this was an actively motile vibrio which appeared on a single slant out of a considerable number inoculated from the first specimen of pus examined. As it grew readily on all mediums and was not encountered again it was discarded as a contamination.

Animal Inoculations.—The first specimen of pus aspirated from the preauricular gland (8 days after admission) was inoculated in the form of a
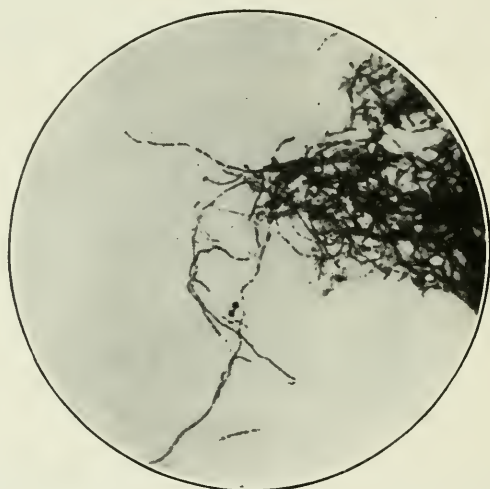
Fig. 2.—The irregularly staining leptothrix isolated on egg yolk from the muscular abscesses of Mouse 1. × 1000.

densely clouded salt solution suspension into the subcutis of a rabbit, guinea-pig, and white mouse. The rabbit and guinea-pig developed slight local reactions which were completely healed in 14 days.

White Mouse 1: Two days after inoculation there was present a somewhat vesicular swelling about 4 mm. in diameter at the site of inoculation. Two days later the local swelling had subsided to about 2 mm. but now the left eye appeared to be suppurating. By the 6th day both eyes were closed and exuded a considerable amount of pus. Smears showed chiefly cocci and solid staining diphtheroid bacilli of the d^2 type. On the 9th day pus from one of the eyes was placed on the conjunctiva of Mouse 2, which remained well during a months' observation. On the 21st day, Mouse 1 died. The spleen and liver were congested and near the tendinous insertions of both knee joints there were muscular abscesses which microscopically showed numerous minute polymorphous gram-negative rods.

The other organs and heart blood showed no bacteria. The bacteria in the muscular abscesses stained poorly with Loeffler's blue or carbol toluidin blue and best after steaming hot Giemsa solution. The abscesses contained a caseous pus which was cultured on a variety of mediums under aerobic, partial tension and anaerobic conditions. No growth appeared in any of the cultures excepting that on a slant of coagulated egg yolk kept under anaerobic conditions and one kept at partial tension, there appeared in 48 hours 30 or 40 colonies, about 0.25 mm. in diameter, raised, dryish, and unpigmented. Microscopically these showed the presence of very delicate rods and filaments (Fig. 2). Attempts to transplant this leptothrix to any of the ordinary mediums failed.

Some of the pus from Mouse 1 injected into Mouse 5 seemed to reproduce the infection.

White Mouse 3: Pus collected on the 17th day from the preauricular gland was rubbed into the left eye. It remained normal for 27 days. The animal was then killed and found to be normal.

White Mouse 4: The animal was inoculated subcutaneously with the same pus used for Mouse 3 and remained well for 27 days. After the animal had been killed examination revealed it to be normal.

Fig. 3.—Mouse 5 inoculated subcutaneously with the abscess pus from Mouse. 1.

White Mouse 5: A mixture of the pus from the eyes and muscular abscess of Mouse 1 was injected subcutaneously. It appeared well and showed no signs of local reaction for 3 days. On the 4th day it appeared to have photophobia and on the 6th day its eyes were closed with double purulent conjunctivitis. It was photographed on the 7th day (Fig. 3). The eyes were still purulent on the 14th day but by the 18th day they were well. The mouse was killed and found free from muscular or other lesions.

Owing to a lack of white mice the following inoculations only could be performed with the culture isolated. A very young white rat, an adult white rat and a single wild mouse (Mus musculus) were inoculated subcutaneously with emulsions of the anaerobic egg yolk culture. They showed no symptoms during 2 weeks observation and when killed were found normal. The egg yolk cultures died after being kept at room temperature for 3 months.

SUMMARY AND CONCLUSIONS

Pus aspirated from the preauricular gland of a case of Parinaud's conjunctivitis and injected subcutaneously in a white mouse, produced an infection, after an incubation period of from 4-5 days, characterized

by purulent conjunctivitis and death. From muscular abscesses found postmortem, a leptothrix was isolated which grew only on slants of egg yolk incubated under partial tension and anaerobic condition. Pus from the mouse injected into a second white mouse reproduced the symptoms but resulted in recovery. The leptothrix isolated was non-pathogenic for white rats and a wild mouse. White mice were not inoculated with the pure culture. Later in the course of the human case a similar organism was isolated on egg yolk directly from the pre-auricular gland. It seems probable, though not certain, that the organism grown by us is identical with that found in sections by Verhoeff. The use of a guinea-pig and a rabbit seemed to rule out the presence of the tubercle bacillus.

THE ACTION OF GLYCEROL ON MICROCOCCI FROM EPIDEMIC POLIOMYELITIS

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During the past year certain micrococci have been demonstrated in the tissues of the central nervous system of persons dead with epidemic poliomyelitis, and there is some evidence at hand indicating that these organisms bear some relation to the disease. Since the causative agent of poliomyelitis has been found to be highly resistant to the action of glycerol it seemed important to determine whether these micrococci possessed the same resisting power for this mild bactericide. The purpose of this brief communication is to present the results of a few experiments bearing on this problem.

In this work cultures of poliomyelitic cocci were compared with different strains of streptococci and pneumococci with reference to their power to resist the bactericidal action of glycerol. The organisms used included 2 strains of micrococci isolated from the spinal cord of poliomyelitis cases, 2 strains of hemolytic streptococci from scarlet fever, 2 strains of Streptococcus viridans from the throats of measles patients, and 1 strain of pneumococcus. Three experiments were made.

In the first experiment the different micro-organisms were grown on blood-agar slants for 24 hours, and the growth of each culture suspended in sterile 50% glycerol. These suspensions were kept at a temperature of 3-5 C., and cultures made at regular intervals. In Exper. 2 the different micro-organisms were grown on blood-agar slants for 24 hours and the surfaces of the slants were then covered with sterile 50% glycerol. These cultures were kept in the refrigerator at a temperature of 3-5 C. and cultures made from them at regular intervals. The results of Expers. 1 and 2 are shown in Table 1.

When the bacteria were grown for 24 hours on blood-agar slants and suspended in sterile 50% or full strength glycerol, they refused to grow after about 3 weeks. The pneumococcus was less resistant than the others, dying in about 2 weeks. One strain of the poliomyelitic coccus remained alive for 30 days. From the blood agar slant

cultures of these organisms which were covered with 50% glycerol positive cultures were obtained after 3 months from all the tubes.

In Exper. 3 the brains and spinal cord of each of two cases of poliomyelitis were placed in 50% glycerol on Sept. 5, and Sept. 9, 1916, respectively. These tissues have been kept in the refrigerator and cultures have been made at monthly intervals since that time. After 15 months both strains of poliomyelitic cocci were alive and easily obtainable by culture from the glycerolated tissues. The organisms obtained in these cultures were similar in morphology and cultural characters to those of the primary cultures. Rabbits injected intravenously with large doses of these cultures were not affected. The primary cultures of both strains obtained at necropsy in the same dosage, however, produced death of rabbits with changes suggestive of those of poliomyelitis.

TABLE 1

The Action of Glycerol on Cultures of Poliomyelitic Cocci and Other Organisms

Organism	Period of Viabilty When Suspended in 50% Glycerol	Period of Viability on Blood-Agar Slant Covered with 50% Glycerol
Poliomyelitic coccus No. 1	30 days	90 days
Poliomyelitic coccus No. 7	21 days	90 days
Streptococcus scarlet fever No. 1	21 days	90 days
Streptococcus scarlet fever No. 2	21 days	90 days
Streptococcus viridans No. 1	21 days	90 days
Streptococcus viridans No. 2	21 days	90 days
Pneumococcus	14 days	90 days

CONCLUSIONS

Poliomyelitic cocci as well as pneumococci, hemolytic streptococci and Streptococcus viridans when suspended in 50% glycerol remain viable for 15-30 days. In the presence of a suitable culture medium such as blood agar, however, cultures of these organisms may remain alive for 90 days.

In two instances poliomyelitic cocci were obtained in culture from the glycerolated nervous system of cases of poliomyelitis after a period of 15 months.

It seems probable then that the micrococci associated with poliomyelitis similar to the virus of the disease, are highly resistant to the bactericidal action of glycerol in 50% solution.

It is interesting to note that ordinary streptococci and pneumococci are also markedly resistant to this germicide.

THE PRODUCTION OF PRECIPITINS BY THE FOWL

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The rabbit is used almost exclusively for the production of precipitins for the identification of blood and other proteins. Of the usual laboratory animals it is regarded as by far the best for this purpose, but of course it is not the only animal that can produce precipitins in large amounts. For example, the serum of persons who have been injected with antidiphtheria serum may contain antihorse precipitins in high concentration for some days. This observation indicates that man may be a good precipitin producer. We have another example in the domestic fowl which responds to the injection of foreign blood or serum with precipitin formation just as regularly and readily as the rabbit, if not more so, as observed by Sutherland and shown also in the experiments about to be described.

The only observer to use fowl precipitins on a large scale in the identification of blood seems to be Sutherland[1] in India, but Ewing[2] was the first to use this animal in such work, and Uhlenhuth[3] also records some special observations on precipitin formation by fowls.

Sutherland describes his method of immunization as follows: "On the first day of treatment the fowl receives 5 c c of the serum of man or a domestic animal, injected into the right wing-vein, after careful disinfection of the site of puncture by means of a pledget of cotton wool soaked in ether. On the 4th day 10 c c are injected into the left wing-vein. On the 8th day 10 c c are injected into the peritoneal cavity When feline serum is used the dosage is 4, 8, and 8 c c, as it is found that the fowls readily succumb to larger doses. On the 22nd or 23rd day the fowl is bled into a sterile Erlenmeyer flask, which is left in the ice-chest for 24 hours. The serum that has exuded from the clot is then decanted, and tested as to its precipitin content's strength and specifity."

[1] Scientific Memoirs of Officers of the Medical and Sanitary Departments of the Government of India, No. 39, 1910. Indian Jour. Med. Research, 1915, 3, p. 216.

[2] Med. News, 1903, 83, p. 871.

[3] Kolle u. Wassermann's Handbuch, 1913, 3, p. 264.

I have followed this method with good result. In many cases a single intravenous injection of 10-20 c c of blood, defibrinated or citrated, or serum may give just as good results, but such injections may result in sudden death. I find further that a single intraperitoneal injection of 20-30 c c of defibrinated blood or serum in most cases is followed by a fairly abundant production of precipitin. In the case of cat and rat blood, however, a single injection is hardly enough, but two or more injections 4-6 days or so apart are necessary to bring the precipitin-content up to a satisfactory titer. As would be expected, pigeon blood has only limited antigenic effect in fowl. Injected intraperitoneally in quantities not exceeding 20 c c cat blood has been without serious ill effects, but it has caused more disturbances than any other kind of blood.

After a single injection of foreign blood or serum there takes place a gradual accumulation of precipitin in the blood of the fowl, the high point being reached about 9-12 days after the injection; before long the precipitin-content begins to fall, the normal or nearly normal level being reached in some cases as early as the 20th day, in other cases not until the 60th day or longer. The curve described by the precipitin under these conditions is a typical antibody curve. When several injections are given, the precipitin titer of the serum fluctuates more or less, usually falling after each injection and rising in a few days somewhat higher than the previous high level. Speaking generally, the best time to bleed to obtain the most potent serum is 9-12 days after the last injection. So far nothing has appeared which would indicate that in the modes of antibody-production the fowl differs in any essential particular from other, in this respect, better studied animals. There is more or less individual variation in the power to produce various precipitins, but it seems to me distinctly less so than in the rabbit and this is particularly true in my experience so far with respect to precipitins for human blood. Of the 27 chickens I have injected with human blood or serum, all but 2 gave a precipitin titer of the serum of 12,000 or higher, i. e., the serum produced precipitate in dilutions of human blood of 1 : 12,000 or more of salt solution. Three of these animals were immunized by Sutherland's method; 9 received single intravenous injections of 12-20 c c of serum or 25 c c of blood; and 15 received single intraperitoneal injections of 20 c c of blood. The two failures were in the last group. From these results it seems quite certain that the fowl is a more reliable and more uniformly liberal producer of antihuman precipitin than the rabbit.

For the production of precipitating serum for practical purposes, roosters rather than hens should be used because the serum of roosters as a rule does not contain nearly so much free fat as hen serum, which often is not clear on that account. Young nearly full-grown animals are preferable.

The blood must be collected in as sterile condition as possible. The serum, withdrawn either after the clotted blood has been standing some hours or after centrifugation, should be wholly free from red corpuscles and should be left standing in the cold box for some days before it is used, in order that whatever spontaneous precipitate forms may have time to settle.

As pointed out by Sutherland and Mitra[4] fowl antiserum may give rise to rather prompt reactions in high dilutions of heterologous serums. They found that such nonspecific reactions would occur especially when antiserum kept in a frozen state had been thawed rapidly, due they suggest to modifications in the electric charge of colloid molecules of the antiserum. If the thawing were effected gradually as at room temperature in cold weather and in the ice-chest in hot weather, nonspecific reactions were not observed. These investigators also found that the growth of bacteria or moulds in the antiserum may lead to nonspecific reactions.

I find that with the usual salt content of about 0.9%, fowl antiserum is liable to give rise to nonspecific reactions even when the explanations advanced by Sutherland and Mitra are not applicable and that precipitate then usually forms also in mixtures of antiserum with 0.9% salt solution only. With uncontaminated serum allowed to remain at room temperature for 2 hours or so after being removed from the icebox, apparently all nonspecific reactions are avoided easily by the simple expedient of making the salt content of all blood or serum dilutions used in the tests 1.9% instead of 0.9%. Consequently in all practical work with fowl antiserum these special precautions should be used to avoid all misleading nonspecific reactions.

Table 1 gives the titers of a number of different kinds of antiserums, most of them developed by a single intraperitoneal injection. The figures on the table as well as on the curves give the highest dilution in 1.8% NaCl solution of laked blood, the salt content having been restored, in which the antiserum produced distinct precipitate after one hour at room temperature.

The tests were made in small tubes by the contact, ring or zonal method, that is to say, small quantities of antigen-dilutions having

• Indian Jour. Medical Research, 1914, 1, p. 707.

been placed in tubes, about 0.1 c c of antiserum is introduced in each tube at the bottom, by means of capillary pipets, so that a well defined plane of contact is formed by the two fluids.

In practically all the instances shown in the table, in which an antiserum was tested with its proper antigen, a layer of precipitate formed
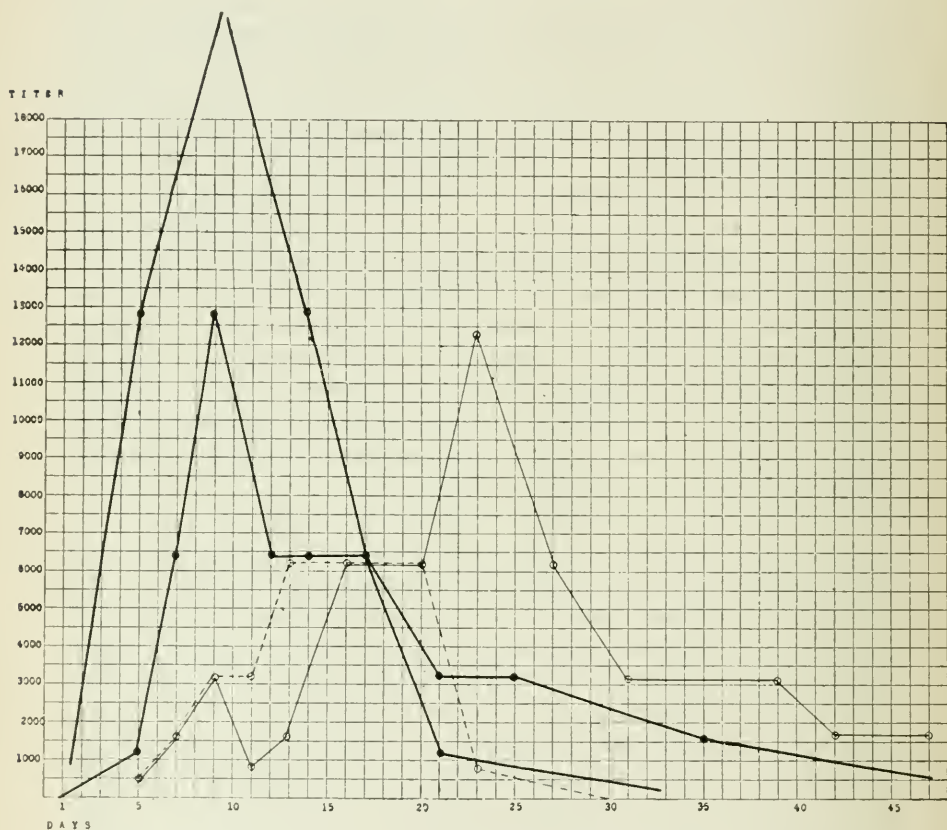
Chart 1.—Precipitin curves in fowls. The heavy black lines show the course of precipitins for human blood in 2 fowls, each injected intraperitoneally with 20 c c of human blood on the 1st day.
The solid fine line indicates the precipitin for rabbit blood in a fowl injected intraperitoneally with 20 c c rabbit blood on the 1st day, and reinjected in the same way on the 10th day. The broken fine line shows the precipitin in a fowl injected intraperitoneally with 20 c c rabbit blood on the 1st day only.

almost at once in all dilutions up to 1 in 1,000, and even higher; and as a rule, in the dilutions recorded as marking the limit of precipitating action, in other words giving the titer of the antiserum, precipitate would appear within 20 minutes. On the other hand, in tests with

blood other than that used in the production of particular antiserum, whatever precipitate formed would form more slowly and with a few exceptions in low dilutions only.

The generally accepted standard of potency for precipitating serum is a reaction within a minute or two in a 1 : 1,000 dilution of its homologous serum or blood in salt solution; and the standard of specificity is that no reaction occurs in any heterologus serum or blood dilution except after 20-40 minutes or longer, and then only in the lower dilutions. Viewed in the light of these standards, the results of my observations establish that it is an easy matter to obtain from the fowl precipitating serum of the strength and specificness required for practical purposes. As pointed out already, this is certainly true

TABLE 1

Precipitin Titers of Fowl Antiserums

Antiserums*	Beef Blood	Cat Blood	Dog Blood	Goat Blood	Guinea-pig Blood	Horse Blood	Human Blood	Rabbit Blood	Rat Blood	Sheep Blood	Swine Blood	1.8% NaCl Solution
Antibeef..........	12800	0	200	6400	0	200	600	0	0	6400	0	0
Anticat...........	0	6400	800	400	200	0	0	0	0	200	0	0
Antidog...........	0	800	6400	200	0	0	400	1600	800	0	0	0
Antigoat..........	6400	0	0	25000	200	200	0	0	200	25000	200	0
Antiguinea-pig.....	0	0	0	0	6400	0	0	0	0	0	0	0
Antihorse.........	200	0	0	200	0	12800	200	0	0	200	0	0
Antihuman serum.	200	0	0	200	0	0	12800	0	0	0	400	0
Antirabbit........	0	0	0	0	0	0	0	6400	400	0	0	0
Antirat...........	0	0	0	0	0	0	200	200	6400	0	0	0
Antisheep.........	800	0	0	1600	0	400	0	0	0	6400	0	0
Antiswine.........	0	0	0	0	0	0	0	0	0	0	12800	0

* All from single injections except cat and rat.

with respect to the most frequently used serum, the antihuman. The only marked exception to the standard of specific limitations of action of precipitating serum are the antibeef and antigoat serums. Each caused precipitates in high dilutions of the blood of the three ruminants tested—beef, goat, sheep. It is rather probable too that if the antisheep serum studied had been of a higher titer, its action on ruminant blood other than sheep would have been more pronounced. Antiruminant rabbit serum also may contain precipitins of high titer for ruminant blood or serum other than used in the immunization. Hence it may be necessary to use special methods in order to identify the blood of a particular ruminant by means of antiserum, either rabbit or fowl, but even so it might prove very difficult to distinguish between sheep and goat blood. In the case of fowl antiserum with too diffuse precipitat-

ing action, Sutherland and Mitra[4] secured good results by dilution of the antiserum with normal fowl serum.

From the results described in the foregoing it is evident that in case of necessity the fowl can be used as a substitute for the rabbit for the production of precipitating serums in general, as has been done by Sutherland in India. Wherever rabbit antiserum is available, however, it perhaps will be best in forensic work to limit the use of fowl antiserum for the present to cases which demand a definite answer to the question whether a given blood spot is rabbit blood. The fowl reacts to a single injection of rabbit blood with the prompt production of antirabbit precipitin of high titer. In two cases it proved easy by means of antirabbit fowl serum to demonstrate that certain blood spots, in one case on a newspaper and in the other on shoes, were due to rabbit blood. To distinguish between hare and rabbit blood it probably would be necessary to use special methods, such as the cross-immunization described by Uhlenhuth, who found that hares develop antirabbit precipitins on repeated injection of rabbit serum.

SUMMARY

The domestic fowl is a prompt, reliable and liberal producer of precipitins, more so than the rabbit. A single intraperitoneal injection of 20 c c of defibrinated blood or serum in most cases in 10-12 days yields a precipitating serum of sufficient strength and specificity for practical purposes.

On account of an unwelcome tendency to give nonspecific reactions, especially on rapid transfer from low to higher temperatures, great care must be exercised in all tests with fowl antiserum, and 1.8% salt solution should be used in making all mixtures and dilutions.

STREPTOTHRIX INTERPROXIMALIS. N. SP. AN OBLIGATE MICROAEROPHILE FROM THE HUMAN MOUTH

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An organism of exceedingly interesting morphology and cultural characteristics has been isolated from the mouth. The mouths from which this organism was isolated would be classed as "habitually unclean mouths with incipient caries and a liberal deposit of salivary calculus."

After having worked with mouth organisms on agar and glucose agar under aerobic and anaerobic conditions, and finding them unfavorable to the growth of the thread forms so common in the oral flora, I substituted Martin's agar[1] and pleuritic fluid, and used the system of partial oxygen tension of Wherry and Oliver[2] during incubation.

Isolation.—Materia alba from the gingival and interproximal portions of the central lower incisors was emulsified in sterile water, by very diligent shaking. Proportionate amounts of this emulsion were inoculated into tubes of equal parts of melted Martin's agar and pleuritic fluid, and the tubes carefully manipulated to make roll tubes. These tubes were then connected by airtight rubber tubing, to cultures of freshly inoculated B. subtilis, and grown at 35 C. (average mouth temperature) for 5 days when they were opened for examination.

A small, round, white, elevated colony was fished and transplanted to slants of the same medium, and grown aerobically, anaerobically and at partial tension. Very slowly, during 5 days, the partial tension culture grew, to form white, slightly raised, rather dry but tenacious colonies. The aerobic and anaerobic tubes gave absolutely no growth.

Subcultures were made on the same medium, and grown under the three varying oxygen pressures, with the uniform result that the partial tension tube gave a growth, while the aerobic and anaerobic tubes were consistently without growth. The colonies were practically invisible until the 4th day, and attained their maximum growth by the 5th or 6th day.

Six consecutive subcultures were made, at the end of 5 days each, from the partial tension tubes, when finally the aerobic tube showed a faint growth. This tube, however, was tightly capped with a rubber hood to prevent drying of mediums, and in this manner a somewhat reduced oxygen pressure may have been produced. This aerobic strain was carried through 2 subcultures, when it died out.

[1] Martin, W. B. M.: Jour. Path. and Bacteriol., 1911, 15, p. 76.
[2] Jour. Infect. Dis., 1917, 20, p. 28.

Subcultures from Martin's medium were carried over to glucose agar with pleuritic fluid, and later to glucose agar (all at partial tension), and the latter medium was used for all stock cultures.

Morphology.—A highly pleomorphic organism, growing in plaquelike colonies of intertwined and matted threads, it varies in its morphology rather consistently with the age of the culture.

1. A 5-day culture shows rather heavy, long, intertwined threads. These threads are about 2-4 microns in width, and vary much in length, probably being broken up in smear preparations, since the colonies are very tenacious. The threads vary from 5-80 microns in length. They are not of a constant diameter, reminding one of a grasshopper's leg. Straight and curved, unbranched fibers are the rule, but occasional branched forms are noted. Many of these fibers are of the same diameter throughout, but an almost equal number present either distal enlargements or segmental indentations, or both. Many of the shorter threads are apparently only fragments of the longer threads, broken in making smears. An occasional knoblike end is noted.

The branching forms, not at all unusual, might be divided into three types: (*a*) Threads ending in two equal branches; (*b*) threads showing one or many lateral knobs, and (*c*) threads showing lateral branching of equal size, but with a very marked constriction at the point of origin (Figs. 1 and 2).

The strain which grew aerobically a short time presented these forms and differed only in the greater number of branching forms.

The ends of the threads were either straight and broken off, slightly rounded, or bulbous, and a few were knobshaped. This picture was quite constant for all 3-5 day cultures on all the mediums.

2. A culture, grown 2 weeks at partial tension, presented an organism of an entirely different form. The involution forms were varied, and fantastic in the extreme. Knobs, commas, heavy crutchlike forms, horns, scrolls, and plain and spotted rods were present. The crutchlike forms were suggestive of the dichotomous branched forms of the previous type; the spotted rods had been suggested by a few forerunners in the 3-5 day culture. Nothing was suggestive of spore formation, except these rounded or ovoid masses in a shell that had been a thread. These masses, however, stained with the greatest ease and subcultures from this type gave healthy growth (Fig. 3).

3. Cultures allowed to remain in the incubator 2 months, at partial tension, showed no increase in growth after the 5th day. Examination showed rod forms, 2 microns by 10-20 microns, some straight, some curved, but none branched, with a poorly staining wall, containing highly staining granules, almost round, usually situated at the ends of the rod forms, and from 2-8 intermediate. I was not successful in subculturing this type (Fig. 4).

Staining.—For routine work, Loeffler's methylene blue, aqueous safranine and gentian violet do well.

By far the greater number of fibers retain the stain by Gram's method, the swollen ends and the swollen portions just adjacent to the indented portions, being stained intensely violet. Some of the threads, throughout their length, take the counterstain. A very considerable number of the threads retain the violet at one end, and progressively take more of the counterstain, as the other end is approached. A few of the fibers, most of them rather short, stained irregularly, with a resultant ground glass appearance.

Type 2 stained very irregularly, and while some forms retained the stain by Gram's method, others took the counterstain. Some forms showed evidences
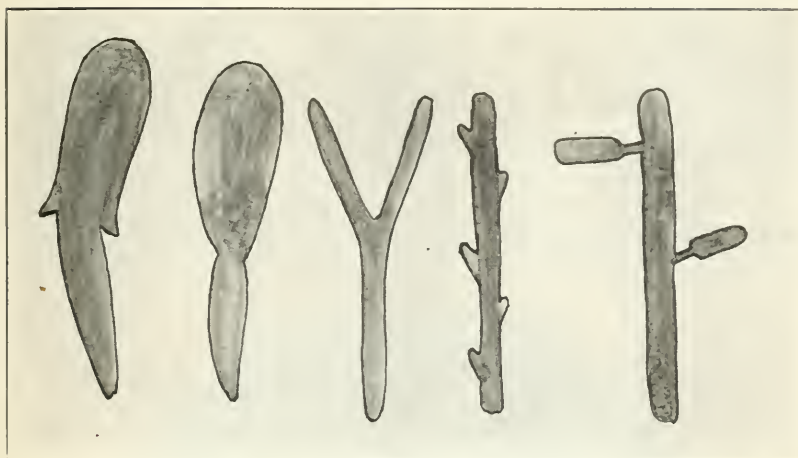
Fig. 1.—Typical forms in a five day culture.

Fig. 2.— Five days' culture.

of both stains, in varying densities. Some forms took practically no stain. The granular type was noticeable for the deep violet the contained granules retained.

Type 3 consisted of a shell which took no stain or a faint counterstain, while the round or ovoid granules retained the violet by Gram's stain. These granules sometimes seemed to be in the form of platelets or plaques, peripherally located in the shell of the fiber.

None of these forms are acid fast, when stained with carbolfuchsin, nor do any give the granulose reaction.

The organism is not motile. It grows not at all anaerobically, with the greatest difficulty aerobically, and rather slowly under a condition of partial oxygen pressure. It is not a chromogenic organism; grows best at 35 C. and
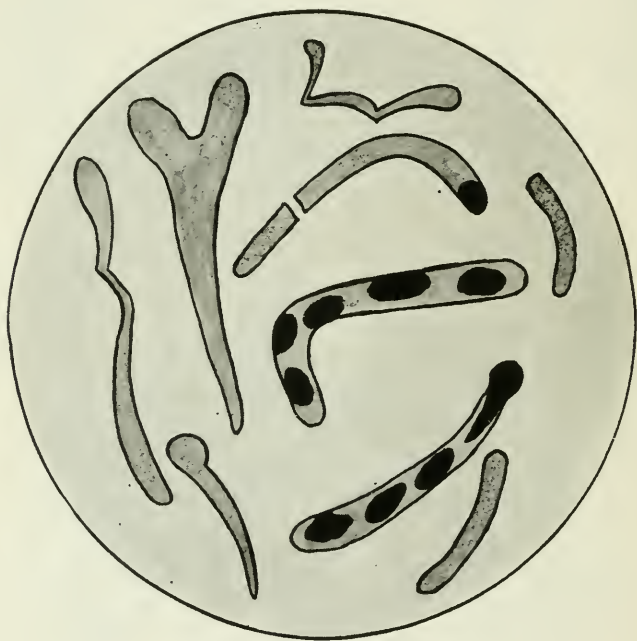
Fig. 3.—Two weeks' culture.

not at all at room temperature. It does not form endospores; does not give rise to any odors on any medium, and is best isolated on a serum agar, though I have once isolated it on dextrose agar. Great difficulty in making subcultures was experienced, due to the tenacity of the colonies. It was later found better to remove the colony entire, emulsify in normal salt solution, and make subcultures from the emulsion. Dextrose agar gives a good and typical growth; maltose agar is almost equally good.

On dextrose agar slant in 2 days a very faint change in the surface of the medium may be noted. In 3 days many pinpoint, white, translucent, hemispherical colonies (like half-pearls) with smooth edge, appear. These colonies grow to pinhead size by the 4th or 5th day, but do not become confluent. After

6 days each colony presents a nipplelike elevation in the center, which has a rounded apex. The pearly white appearance of the colonies continues, the surface being smooth and glistening, in contradistinction to dry and chalky. The colony grows slightly in size up to this time, the largest colony attaining a diameter of only 1.5-2 mm., but growth discontinues from this time on. By transillumination the colonies are dark brown, with a lighter halo.

Dextrose agar stab, under aerobic conditions, gives no deep growth, nor any surface growth, but a faint growth in the upper 5 mm. of the stab.

Plain agar slant gives the same as the dextrose agar, except that the growth is not so profuse, and that the individual colonies are very minute.

Maltose agar yields results identical with those on dextrose agar.

Fig. 4.—Two months' culture.

On Loeffler's blood serum, the usual growth takes place, but without liquefaction.

On blood agar, typical growth results, without hemolysis.

Neither on gelatin nor potato does growth take place.

In neither sugar-free broth, glycerol broth, starch broth nor milk, after repeated efforts, could growth be obtained.

In dextrose litmus broth, there is no cloudiness, no scum, no precipitate, nor any visible evidence of growth at any time, though the organism was later recovered from the medium. A slight acidity was noticed after 48 hours, which increased until, after 4 days, the acidity was marked.

On maltose litmus broth a slight acidity in 2 days; marked acidity in 4 days.

In lactose litmus broth, no acidity, even after 10 days.

In galactose litmus broth, slight acidity after 2 days, with slight increase after 4 days.

In saccharose litmus broth acid production after 2 days was definite and after 4 days, marked.

The organism, during 5 days' incubation, ferments the sugars with a proportionate ease and quantity of acid expressed in the following order: dextrose, saccharose, maltose, galactose, but lactose not at all.

This organism differs from Goadby's Streptothrix buccalis in many important details. The inability of this organism to grow either under aerobic or anaerobic conditions, the presence of dichotomous branching, an absence of the "white powdery gonidia," on the colonies, its inability to grow at room temperature and liquefy gelatin, and its lack of growth on potato and in milk, serve to differentiate it from Streptothrix buccalis, the only organism it resembles. I have, therefore, tentatively, for the purpose of description and study, named it Streptothrix interproximalis.

CONCLUSIONS

Streptothrix interproximalis has its natural habitat in the unclean mouth, and may play some part in the etiology of dental caries or chronic peridental inflammation.

It is a highly pleomorphic organism, with fairly constant types representing 5 days', 3 weeks' and 2 months' cultures.

It grows only under conditions of reduced oxygen pressure.

It is worthy of study, chiefly because it indicates by its strict cultural requirements, that a method for cultivating many of the heretofore uncultivated organisms, particularly of the oral flora, may have been found.

THE PNEUMOCOCCIDAL POWER OF RABBIT SERUM AFTER THE ADMINISTRATION OF ETHYLHYDRO-CUPREIN HYDROCHLORID, QUININ AND UREA HYDROCHLORID, AND OTHER CINCHONA DERIVATIVES

STUDIES IN PNEUMONIA, VII

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Previous studies by Solis-Cohen, Kolmer, Heist, and Steinfield on the mechanism of the action of cinchona derivatives in pneumonia of man have indicated the highly specific pneumococcidal action of these salts, both in vitro[1] and in vivo,[2] as well as an accelerating action on leukocytosis and phagocytosis in vitro.[3] In this article I wish to present briefly the results of studies of the pneumococcidal action of the serum of rabbits after the administration of ethylhydrocuprein hydrochlorid, quinin and urea hydrochlorid, and other cinchona compounds.

Wright[4] was first to observe the pneumococcidal action of the serum of human beings and mice treated with ethylhydrocuprein. He calls attention to the negative results obtained with rabbits. Scott[5] likewise obtained negative results with ethylhydrocuprein in the treatment of pneumococcus infections in rabbits. Boecker[6] thoroughly investigated this problem and also obtained negative results. Recently Moore and Chesney[7] in an extensive study on the use of ethylhydrocuprein in the treatment of acute lobar pneumonia in man pointed out the necessity of repeated doses of the drug before any bactericidal action of the serum can be demonstrated. With this suggestion in mind and working with derivatives of cinchona less toxic than ethylhydrocuprein, it was hoped that increased pneumococcidal action of the serum of normal rabbits could be demonstrated.

[1] Jour. Infect. Dis., 1917, 20, 272.
[2] Ibid., p. 313.
[2] Ibid., p. 333.
[4] On Pharmaco-therapy and Preventive Inoculation Applied to Pneumonia, 1915.
[5] Jour. Path. and Bacteriol., 1914-1915, 19, p. 130.
[6] Ztschr. f. Immunitätsforsch., O., 1915, 24, p. 148.
[7] Arch. Int. Med., 1917, 19, p. 611.

Normal healthy rabbits weighing from 1,200-3,000 gm. were used. The drugs were ethylhydrocuprein hydrochlorid, quinin and urea hydrochlorid, quinin chlorhydrosulphate, and quinin dihydrobromid. For oral administration the proper dose of drug was dissolved in distilled water and given by means of the stomach tube. Physiologic saline solution was used as the menstruum for intravenous injections. Bleedings were made from the ear vein before administration of the drug and at intervals from half to two or more hours thereafter. All specimens were allowed to remain in the icebox over night to allow the serum to separate. The clear serum was used after sterilization by heating in a waterbath at 56 C. for one-half hour.

The organism employed was a virulent Type 1 pneumococcus from the Rockefeller Institute. Sixteen hour-old cultures in blood-glucose broth were used. The technic (Expers. 1 to 3) was that described by Moore and Chesney;[7] later we used the plating method of Kolmer.[8]

[8] Jour. Infect. Dis., 1917, 20, 293.

The protocols of a few typical experiments are given as illustrative of the results.

Exper. 1.—Single lethal dose of ethylhydrocuprein hydrochlorid by oral administrations. A rabbit weighing 2,200 gm. was given ethylhydrocuprein hydrochlorid by stomach tube in a dose of 0.4 gm. per kilo. The animal died 3½ hours after administration of the drug. No pneumococcidal action was demonstrated in the serum of the heart's blood taken immediately after death.

Exper. 2.—Single lethal intravenous dose of ethylhydrocuprein hydrochlorid. A rabbit weighing 1,500 gm. was given ethylhydrocuprein hydrochlorid intravenously in a dose of 0.02 gm. per kilo. The animal died immediately after injection with typical convulsion of cinchona poisoning. The serum obtained from the heart's blood showed no pneumococcidal action.

Exper. 3.—Single tolerated doses of quinin and urea hydrochlorid and quinin chlorohydrosulphate by oral administrations. Two rabbits weighing 1,000 and 1,700 gm. were given quinin and urea hydrochlorid, and quinin chlorohydrosulplate, respectively, by stomach tube in doses of 0.5 gm. per kilo of weight. The animals showed slight cinchonism but recovered. No pneumococcidal action was observed in serum obtained 5 hours after the injection.

Exper. 4.—Repeated doses of quinin and urea hydrochlorid, and quinin chlorohydrosulphate by oral administrations. Two rabbits weighing 1,500 and 3,000 gm. were given quinin and urea hydrochlorid by stomach tube in doses of 0.5 and 0.4 gm. per kilo, respectively. Three hours later each was bled and then given a second dose of 0.1 gm. per kilo by stomach tube. The second rabbit died immediately afterward and the heart blood was collected. The first rabbit was bled 2 hours later and again given a dose of the drug corresponding to 0.1 gm. per kilo and again bled 2 hours after the last dose. The animal was found dead the next day. None of the serums were found to exert any pneumococcidal action when allowed to act for 24 hours at 37 C. in the dark on 1 c c of a culture of pneumococcus Type 1 in dilutions ranging from 1 : 100 to 1 : 10,000.

Two other rabbits receiving quinin chlorohydrosulphate behaved in a similar way.

Exper. 5.—Repeated intravenous injections of quinin chlorohydrosulphate, quinin and urea hydrochlorid and quinin dihydrobromid. Three rabbits weighing 1,700, 1,500 and 1,200 gm. were given quinin chlorohydrosulphate, quinin and urea hydrochlorid and quinin dihydrobromid, respectively, in initial doses of 0.01 gm. per kilo. Thirty minutes later bleedings were made. Three hours later a second dose of 0.01 gm. per kilo was given to each and bleedings were made 2 hours after the injections. The serum was at no time pneumococcidal.

The experiments indicate clearly that a single large tolerated dose, a fatal dose, or repeated tolerated doses of varying cinchona derivatives administered either by intravenous or oral route do not serve to render pneumococcidal the serum of rabbits. These results are in accord with those of Wright, Scott and Boecker.

In explanation Scott demonstrated that the destructive power of the rabbit liver for quinin alkaloids is 10 times greater than that of the guinea-pig or mouse. Grosser[9] showed the same holds true for the liver of the cat. Lippmann[10] observed that ethylhydrocuprein exerts its pneumococcidal action in vivo only in the presence of leukocytes. Boecker cites the work of Morgenroth and Ginsberg and suggests that the red blood cells have a special affinity for optochin. This suggestion we find borne out in the quantitative chemical studies of Baldoni[11] who finds that after the administration of quinin to animals by subcutaneous or oral routes, more quinin is demonstrable in the erythrocytes than in the serum. We have elsewhere[12] called attention to the extreme hemolytic power of isotonic solutions of various quinin salts as an index of their affinity for the red blood cells and their property of precipitating serum (and other colloidal solutions) as an index of their lack of affinity for the latter.

SUMMARY

No pneumococcidal action of rabbit serum could be demonstrated after a single large tolerated dose, after a single fatal dose, after repeated injections of tolerated doses either by oral or by intravenous routes of various cinchona derivatives including ethylhydrocuprein hydrochlorid, quinin and urea hydrochlorid, quinin dihydrobromid, and quinin chlorohydrosulphate.

A review of the literature indicates that the liver of the rabbit has a high destructive action on quinin alkaloids and that both leukocytes and red blood cells (but not serum) have a marked affinity for cinchona compounds.

9 Biochem. Ztschr., 1908, 8, p. 98.
10 Ztschr. f. Immunitätsforsch., O., 1915, 24, p. 107.
11 Arch. di Farmacol. sper., 1912, 13, p. 324; abstracted in Zentralbl. f. Biochem. u. Biophysik, 1912-1913, 5, p. 315.
12 Jour. Infect. Dis., 1918, 22, p. 476.

FURTHER STUDIES ON BACTERIUM ABORTUS AND RELATED BACTERIA

I. THE PATHOGENICITY OF BACTERIUM LIPOLYTICUS FOR GUINEA-PIGS

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The organism causing infectious abortion has been known as Bacillus abortus. But in accordance with the recommendation of the Committee of the Society of American Bacteriologists on Characteriatzion and Classification of Bacterial Types,[1] the family Bacillacea, including the genus Bacillus, is characterized by the production of endospores. Such a classification would exclude the abortion organism from that genus. The committee suggests that it be included in the genus Bacterium. Accordingly the name of the abortion organism becomes Bact. abortus. The generic name Bacillus has hitherto also been applied to two other organisms discussed in this series of papers which are similar in morphology to Bact. abortus. To be consistent in nomenclature therefore, the two organisms are called, respectively, Bact. bronchisepticus and Bact. melitensis.

In earlier publications [2,3] it has been shown that there is commonly present in large numbers in milk, a variety of bacterium which agrees with Bang's[4] description of Bact. abortus, but which differs markedly from the virulent strains of Bact. abortus which were available for comparison. The common milk variety was designated Bact. abortus var. lipolyticus[5] because of its ability to break down the butterfat to fatty acids. The question arose as to whether it might possess disease-producing properties. Accordingly, a series of inoculation and feeding experiments with guinea-pigs was conducted to determine the point in question.

Inoculation Experiments.—The inoculation of the animals and postmortem examinations were made by Dr. G. M. Potter, formerly of the Pathologic Division of this Bureau. Thirteen guinea-pigs were inoculated with 2 c c of broth culture of Bact. lipolyticus. Three were inoculated with milk known to be naturally infected with the organism. They received 2½, 5, and 7½ c c, respectively. All inoculations were made intra-abdominally. Altogether, 16 guinea-pigs were inoculated.

After the death of the animals, cultures were made from the lungs, heart blood, kidneys, liver, and spleen, and in some cases from other organs, in plain-infusion agar and in agar to which 10% of sterile, raw cow's-blood serum had been added. Slope, shake, and plate cultures were made.

The results of the experiments were complicated by a contagious infection with a small, rod-shaped organism, similar in morphology to the abortion

[1] Jour. Bacteriol., 1917, 2, p. 546.
[2] Evans: Jour. Infect. Dis., 1916, 18, p. 437.
[3] Evans: Jour. Bacteriol., 1917, 2, p. 185.
[4] Ztschr. f. Tier., 1897, 1, p. 241.
[5] The name Bact. abortus var. lipolyticus is unwieldy. Although the organism is related to Bact. abortus, it is no more closely related to that organism than are several other types of bacteria which bear distinct species names. Therefore the fat-splitting bacterium will be called Bact. lipolyticus in this series of papers.

organism, but readily distinguishable by its power to produce gas in the agar shake cultures. Two of the inoculated animals died as a result of infection with the gas-producing organism. Of the remaining 14 animals, 7 died between the 49th and 67th day after inoculation, and from 3 of those animals which died, there was isolated a small, rod-shaped organism which resembled pathogenic strains of Bact. abortus. It was isolated from the lungs of one animal that died of pneumonia, 67 days after inoculation; from the heart blood of one, and from the lungs of another, both of which died 52 days after inoculation, with no gross lesions showing at postmortem.

Feeding Experiments.—Since the udder of the cow is the natural habitat of Bact. lipolyticus, the possibility suggested itself of infection by ingestion of large numbers of the organisms with the milk. Therefore a series of feeding experiments with guinea-pigs was planned. At the beginning of the feeding the weights of the animals ranged from 200-375 gm. The guinea-pigs soon learned to like the milk, which was poured over their oats, and they took considerable quantities of it. The milk was from a cow which was known to have eliminated large numbers of the organism for 2 years preceding the first feeding experiment. During the period of the experiment the numbers varied from a few thousand per cubic centimeter on some days to 70,000 on the day the largest count was made. Milk from the same cow was pasteurized for feeding control animals. At the time the first group was fed with the naturally contaminated milk, another group of guinea-pigs was fed with a pure culture isolated from the raw milk. The organisms were grown in mass on serum agar in plate cultures. A heavy growth was obtained by flooding the surface of the agar with a whole-milk culture and then incubating for 2 days. A water suspension containing the growth from 2 or 3 plates was fed with the oats every day to each guinea-pig.

In the first feeding experiment there were 3 groups of guinea-pigs, with 4 in each group. One group was given pasteurized milk, the second raw milk, and the third the pure culture. After 70 days of feeding, the experiment was discontinued, for at that time, of the groups which had been receiving raw milk or pure culture, there was only one animal living, whereas three of those fed with pasteurized milk were in good condition, one having died as a result of infection with the gas producer. No definite conclusions could be drawn from this first feeding experiment, for no results were obtained by incubating cultures from the organs. The circumstances indicated, however, that the ingested bacteria may have caused the death of the animals.

In the second feeding experiment there were 3 groups of guinea-pigs, with 10 in each group. One group was fed with pasteurized milk, the second was fed with raw milk, and the third group was fed with inoculated milk. The pasteurized and the raw milk was from the same source, and treated in the same manner as in the first experiment. The inoculated milk was sterilized milk which had been inoculated with a pure culture isolated from the raw milk. The milk was incubated until it contained about the same number of bacteria as the raw milk.

Of the 10 animals fed with pasteurized milk, 6 died as a result of infection with the gas produced before the experiment was discontinued, after 99 days feeding. The remaining 4 animals were then killed, and postmortem examinations were made. No gross lesions were found, and cultures from the organs showed no growth, or a few scattered colonies of contaminating bacteria.

Of the 10 guinea-pigs fed with raw milk, one died as a result of infection with the gas producer; one died as a result of a ruptured stomach; one died on the 39th day with no gross lesions, and with no growth developing in cultures made from the organs; one died of pneumonia on the 62nd day, and a small rod-shaped organism resembling Bact. abortus was isolated from the lungs. This was the

same organism which had been isolated from the organs of 3 of the inoculated guinea-pigs. It was isolated also from the lungs of one, and from the lungs and spleen of another of the guinea-pigs fed with raw milk which were killed on the 99th day, with no gross lesions appearing in postmortem examination. No results were obtained from the remaining 4 animals of this group, 3 of which were killed on the 79th, and 1 on the 99th day.

Of the 10 guinea-pigs fed with inoculated milk, 4 died as a result of infection with the gas producer; 1 died of pneumonia on the 94th day, but no growth could be obtained in cultures inoculated with the lungs and other organs. The remaining 5 animals were killed, 2 on the 79th, and 3 on the 99th days. The organs of all 5 appeared normal, and no growth could be obtained in cultures inoculated with them.

DISCUSSION

Organisms resembling Bact. abortus were isolated from the organs of 3 of the 16 guinea-pigs that were inoculated with Bact. lipolyticus, either in pure culture or in naturally contaminated milk. These 3 animals died between the 52nd and 67th days after inoculation. Four more of the 16 inoculated guinea-pigs died between the 49th and 67th days, but no cultures were obtained from the organs. The time of death of the animals agreed with the time required for Bact. abortus to develop lesions in guinea-pigs, as reported by Fabyan.[6] This investigator states that the lesions in guinea-pigs inoculated with Bact. abortus usually appear between the 3rd and 6th week, the acute changes extending over a period of 10-20 weeks, after which reparative processes appear.

Twenty-eight guinea-pigs were fed with Bact. lipolyticus in naturally contaminated milk, or in inoculated sterile milk, or in mass of pure culture. The organism resembling Bact. abortus which was isolated from the organs of 3 of the inoculated animals was isolated also from the organs of 3 of the animals which had been fed, from the lungs of 1 which died of pneumonia on the 62nd day and from the apparently normal organs of 2 guinea-pigs which were killed on the 99th day.

These results were misleading, for it is now known that the abortion-like organism isolated from 3 of the inoculated animals and from 3 of the fed animals was another contagious infection which complicated the experiments. It was apparently a mere matter of circumstance that the infection prevailed in the cages with inoculated and fed animals, whereas the cages with the control animals escaped the infection.

The similarity of the infectious organism, which has been identified as Bact. bronchisepticus, with pathogenic strains of Bact. abortus will

⁶ Jour. Med. Research, 1912, 26, p. 441.

be demonstrated in the second paper of this series. They resembled one another so closely that it was hypothetically assumed that the lipolyticus variety of Bact. abortus had acquired the characteristics of pathogenic strains during the several weeks or months of life in the guinea-pig's bodies. This led to an erroneous conviction when cultures of the lipolyticus variety and of the recovered abortion-like bacteria were submitted to another investigator for serologic tests, and it was reported that the blood of a rabbit immunized with a strain of the lipolyticus variety strongly agglutinated the strains obtained from the guinea-pig organs. Subsequently, it was found that these results were due to an error in the reading of labels, and that the rabbit had not been immunized with the lipolyticus strain but with Bact. bronchisepticus.

If those animals are eliminated which died of one or the other of the contagious infections, including with those from whose organs the causal organism was isolated, 2 which died at about the same time but from whose organs no cultures were obtained, there remain 7 guinea-pigs which were inoculated with pure culture of Bact. lipolyticus. Four of these were killed; 2 on the 48th day, 1 on the 67th and 1 on the 70th day after inoculation. The appearance of all organs was normal, and no growth could be obtained in cultures heavily inoculated with the organs. The remaining 3 animals showed no signs of any ill effects resulting from the inoculations through several months of observation.

Eliminating in the same manner from the 28 guinea-pigs which were fed with Bact. lipolyticus those animals which died with one or the other of the contagious infections as the proved or suspected causal agent, there remain to be considered 12 guinea-pigs. Seven of the twelve were fed with raw milk. Three of these were killed on the 79th day, and 3 were killed on the 99th day. The organs appeared normal at post mortem, and the ingested organism could not be recovered. The other guinea-pig fed with raw milk was not killed. It never showed any ill effects resulting from the feeding. Five of the 12 guinea-pigs were fed with inoculated milk. Two were killed on the 79th, and 3 on the 99th days. The organs of these animals also appeared normal, and the ingested organism could not be recovered.

Although these experiments do not demonstrate the harmlessness of Bact lipolyticus as clearly as could be desired, due to the complications with the two contagious infections, no evidence was found to show that it is pathogenic for guinea-pigs.

FURTHER STUDIES ON BACTERIUM ABORTUS AND RELATED BACTERIA

II. A COMPARISON OF BACTERIUM ABORTUS WITH BACTERIUM BRONCHISEPTICUS AND WITH THE ORGANISM WHICH CAUSES MALTA FEVER

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The similarity between Bacterium bronchisepticus and Bacterium abortus in their growth on potato, and in their inability to attack sugars has been pointed out by Smith.[1] So far as the writer is aware, no further attention has been called to the similarity between the two organisms. From a biologic standpoint the resemblance is of interest; and since, as McGowan[2] has shown, Bact. bronchisepticus causes epizootics among laboratory animals, and during an epizootic may inhabit the organs of normal animals, the resemblance between the two organisms has a practical significance in the possibility of their confusion when isolated from experimental animals.

Certain facts as stated in the literature concerning another pathogenic organism — that causing Malta fever — suggested that a comparison with Bact. abortus might also be of interest. The Malta fever organism was discovered by Bruce.[3] He examined stained smears from the spleen of human subjects in fatal cases, and in every case found minute organisms which he called micrococci. These were later proved to be the causal organism, and the name Micrococcus melitensis was given. In fresh material obtained from infected individuals this organism is said to appear invariably in the coccus form, but under artificial cultivation bacillary forms are reported to be common, and these have been regarded as involution forms. Some of the most recent textbooks, however, have called the organism Bacillus melitensis. The descriptions of the Malta fever organism suggested a similar morphology to that of Bact. abortus which is described as a

[1] Jour. Med. Research, 1913, 29, p. 291.
[2] Jour. Path. and Bacteriol., 1911, 15, p. 373.
[3] Practitioner, London, 1887, 39, p. 161.

"cocco bacillus," with the coccoid forms occurring commonly in pathologic material, and with distinct bacillary forms commonly found in cultures.

The habitats of Bact. abortus and the Malta fever organism also are very much the same. An extensive investigation was made by the British Commission on Mediterranean Fever,[4] which reported that the disease was propagated by goats. The English investigators were able to cultivate "Micrococcus melitensis" from the milk of 10% of the goats supplying milk to various parts of Malta. Judged by the serum reactions, they concluded that 41% of the goats on that island were infected. They were able to infect monkeys and goats by feeding with cultures isolated from the milk, or by feeding the infected milk itself. Schroeder and Cotton[5] found by inoculating cows' milk into guinea-pigs that about 14% of the samples tested were infected with Bact. abortus. In a recent publication Fleichner and Meyer[6] report that as a result of inoculating guinea-pigs with certified milk they conclude that "Bact. abortus is, for all practical purposes, always present in the certified milk produced in the San Francisco Bay regions." Thus it has been shown that the Malta fever organism is a common infection of goats' milk on the Island of Malta, and Bact. abortus is a common infection of cow's milk in this country.

The three organisms, Bact. abortus, Bact. bronchisepticus, and the Malta fever organism, will be described in detail in order that they may be compared.

BACTERIUM ABORTUS

A large number of strains of Bact. abortus which served for this study were obtained from the Pathological Division of this bureau. They had originally been isolated from pathologic material. A few of the strains were isolated from milk. Only typical pathogenic strains were considered. The criterion for judging a culture as typical was agglutination in a high dilution of Bact. abortus immune serum. Only strains which reacted positive to this test in a 1:1,280 dilution or higher of the serums which served for this study were considered. All strains accepted as typical according to this test agreed in practically all cultural and biochemical tests. The author is indebted to Dr. John M. Buck of the Pathological Division of this Bureau for the inoculation of the animals and the postmortem examinations reported in this paper, and for the antiserums used in the agglutination tests.

Morphology.—Bact. abortus is a short, slender, pleomorphic rod with rounded ends, whose form is influenced by the medium in which it was grown. The cells are sometimes so short as to appear coccoid. The width is about 0.5 mikrons,

[4] Reports of the Commission on Mediterranean Fever, 1906, London.

[5] Twenty-eighth annual report of the Bureau of Animal Industry, Department of Agriculture, 1911, p. 139.

[6] Am. Jour. Dis. Child., 1917, 14, p. 157.

the length varies from this dimension to 2 mikrons. (Mohler and Traum[7] report that it may be as long as 3 mikrons.) The morphology of the organism from the condensation water of a 24-hour culture on agar slope is given in Figure 1. Bact. abortus is nonmotile. It does not form spores.

Staining.—Bact. abortus is readily stained with the ordinary dyes, but it is negative to Gram's stain.

Cultural Characteristics.—The first growth of a strain transferred from pathologic material to artificial media is often difficult to obtain. Growth is favored by incubation in a closed jar with a culture of Bacillus subtilis. Glycerol agar or serum agar serves well for such a strain, but after the strain has become accustomed to artificial conditions growth is abundant on all the ordinary media. An infusion agar slope culture of a readily growing strain shows an opalescent growth after 24 hours' incubation, which becomes heavier during the next day or two. It is a lustrous, moist growth with a sharply defined margin. Crystals begin to form in the agar after 5 or 6 days' incubation.

On agar plates after 2 days' incubation colonies like tiny dew drops appear on the surface of the agar. They gradually become opaque as they continue to increase in size during about 10 or 12 days' incubation when finally the largest colonies attain a diameter of about 6 mm. In the depths of the agar there are two kinds of colonies; small, bluish-white, circular colonies about ⅓ mm. in diameter, and opaque, lemon-shaped colonies about ⅓ mm. long.

In agar shake cultures there is an abundant surface growth, but no growth beneath the surface. The agar just beneath the surface growth is rendered white and opaque.

In broth cultures a faint clouding is visible after 1 day's incubation. During the next day or two the clouding becomes heavier, but the broth never becomes heavily clouded. There is no surface ring or pellicle. After several days a sediment begins to precipitate.

In litmus milk the only change is a slight alkalinity apparent after several days' incubation; this reaction never becomes pronounced.

On potato there appears a slight glistening growth of a brownish color. After several days' incubation the potato itself takes on a brownish tinge.

Biochemical Reactions.—Bact. abortus does not attack the sugars nor any of the other commonly used fermentable test substances. In broth cultures there is a reduction of the hydrogen-ion concentration equal to about 0.7 or 0.8 P_H. This reaction is fairly definite and characteristic. The initial hydrogen-ion concentration of the broth may vary over quite a wide range in either direction from the neutral point without affecting the results. Both urea and asparagin are decomposed with the production of ammonia, but the reaction in asparagin medium is often slight. In some cultures there is a slight reduction of nitrates to nitrites; other cultures show no reduction. Indol is not produced in tryptophan medium. Gelatin is not liquefied. The biochemical reactions of two of the strains are given in Table 1. Strain w1 was from an aborted fetus. Strain aap was from milk.

BACTERIUM BRONCHISEPTICUS

Twenty-three strains of Bact. bronchisepticus have come under observation. Twenty-one of the strains were isolated from infected guinea-pigs. One strain obtained from the Pathological Division was isolated from the lung of a dog sick with distemper, and one strain was obtained from Dr. Theobald Smith. All of the 23 strains gave the same reactions to the cultural and biochemical tests.

[7] Twenty-eighth annual report of the Bureau of Animal Industry, U. S. Department of Agriculture, 1911, p. 147.

Morphology.—Bact. bronchisepticus is also a short, slender, pleomorphic rod with rounded ends, whose form is influenced by the medium in which it grows. In the tissues, and sometimes in artificial mediums, it has more or less of a coccal form. McGowan[2] reports that the width is 0.4-0.5 mikrons, and that the length varies from 0.5-2.3 mikrons. The morphology of the organism from the condensation water of agar slope is shown in Figure 2. In the stained smear it cannot be distinguished from Bact. abortus, but Bact. bronchisepticus is motile. No spores are formed.

Staining.—The organism is readily stained with ordinary dyes, but is decolorized by Gram's stain.

Cultural Characteristics.—Bact. bronchisepticus is easily cultivated from infected tissues. McGowan[2] thus describes its growth in agar plate cultures: "After 24 hours, all that is seen is a number of discrete dew points. These enlarge very rapidly during the next 24 hours, becoming as large as pinheads. They are raised above the surface, are regular hemispheres in shape, and have an

TABLE 1

A COMPARISON OF THE BIOCHEMICAL REACTIONS OF BACT. ABORTUS WITH THOSE OF BACT. BRONCHISEPTICUS AND BACT. MELITENSIS

Species	Strain	Reaction in Litmus Whole Milk	Fermentation of				
			Dex-trose	Lac-tose	Saccha-rose	Mal-tose	Man-nite
Bact. bronchisepticus....	vl	Decidedly alkaline	—	—	—	—	—
Bact. bronchisepticus....	wy	Decidedly alkaline	—	—	—	—	—
Bact. abortus............	wl	Faintly alkaline	—	—	—	—	—
Bact. abortus............	aap	Faintly alkaline	—	—	—	—	—
Bact. melitensis..........	yf	Faintly alkaline	—	—	—	—	—
Bact. melitensis..........	aay	Faintly alkaline	—	—	—	—	—

opaque, white, porcelainous look, with an opalescent sheen." The growth is more rapid than that of Bact. abortus, and after several days' incubation the largest colonies attain a diameter of about 8 mm., which is somewhat larger than the largest colonies of Bact. abortus. Otherwise the agar plate cultures of the two organisms cannot be distinguished. Likewise on agar slopes the cultures of Bact. bronchisepticus grow more rapidly, so that at the end of 24 hours their growth is heavier than that of Bact. abortus. Afterward the cultures of the two organisms on agar slope cannot be distinguished. Crystals appear in the agar after several days' incubation, just as they do in agar cultures of Bact. abortus. In agar shake cultures the growth of Bact. bronchisepticus is identical with that of Bact. abortus.

In broth cultures Bact. bronchisepticus can be distinguished from Bact. abortus by a heavier clouding of the medium. This difference is apparent in 24-hour cultures, as well as in cultures of several days' incubation. The Bact. bronchisepticus cultures can also be distinguished by a faint, broken film which covers part of the surface.

In litmus whole milk Bact. bronchisepticus can be distinguished from Bact. abortus by a decidedly greater alkalinity of the medium. The alkalinity appears on the surface of the cream layer in a 24-hour culture. The milk beneath the cream layer becomes alkaline slowly, but in a week-old culture the reaction is pronounced.

On potato there is a glistening growth of a brownish color, somewhat heavier than the growth of Bact. abortus.

Biochemical Reactions.—Like Bact. abortus, Bact. bronchisepticus does not attack the sugars nor the other commonly used fermentable test substances. In broth cultures there is a reduction of the hydrogen-ion concentration equal to about 2.0 PH. Compared with Bact. abortus the more intense alkaline reaction of Bact. bronchisepticus is a definite point of distinction. But the two organisms cannot be distinguished by their other biochemical reactions. Bact. bronchisepticus decomposes urea and asparagin with the production of ammonia. The majority of strains do not reduce nitrates to nitrites; there is no production of

TABLE 1—*Continued*

A COMPARISON OF THE BIOCHEMICAL REACTIONS OF BACT. ABORTUS WITH THOSE OF BACT. BRONCHISEPTICUS AND BACT. MELITENSIS

Decomposition of		Reaction in			Reduction of Hydrogen-ion Concentration in Broth Cultures Recorded in PH Values
Urea	Aspar-agin	Nitrate Broth	Gela-tin	Tryptophan Medium	
+	+	—	—	—	1.9
+	+	—	—	—	2.2
+	+	—	—	—	0.8
+	+	Slight	—	—	0.7
+	Faint	Slight	—	—	0.7
+	Faint	—	—	—	0.8

indol in tryptophan medium; gelatin is not liquefied. In Table 1 the biochemical reactions of Bact. bronchisepticus can be compared with those of Bact. abortus.

Agglutination Reactions.—The serum used for the agglutination tests was obtained from a cow which had been inoculated with Bact. abortus many times by the investigators of the Pathological Division for the purposes of another experiment. Her serum agglutinated Bact. abortus suspensions in higher dilutions than that of naturally infected cows. Bact. bronchisepticus was agglutinated in low dilutions of this antiserum. In Table 2 the reactions of 6 strains are given, together with the reactions of typical strains of Bact. abortus. Strain yc was the only one of the collection which failed to give an agglutination reaction in low dilutions of the serum. This strain was the one which was obtained from Dr. Theobald Smith. Morphologically, culturally, and biochemically it was identical with our strains. Strain xb, which was isolated from the lung of a dog sick with distemper reacted in the same manner as the strains isolated from guinea-pig organs. The agglutination of Bact. bronchisepticus in dilutions of 1 : 40 or 1 : 80 of this Bact. abortus antiserum readily distinguishes that organism from Bact. abortus, which was agglutinated in dilutions of 1 : 1,280 or higher of the same serum.

Fig. 1.—Bact. abortus.
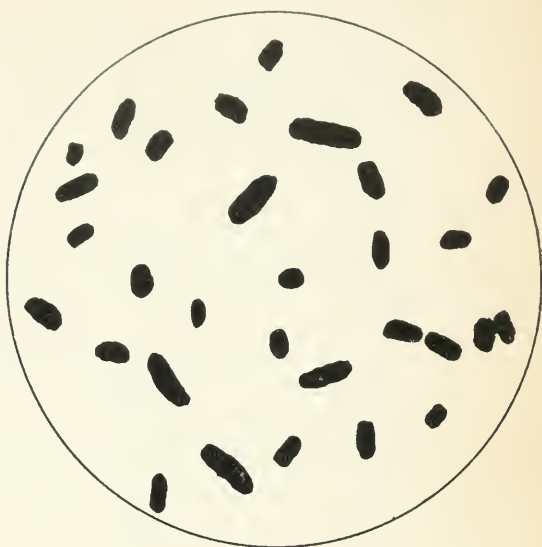
Fig. 2.—Bact. bronchisepticus.
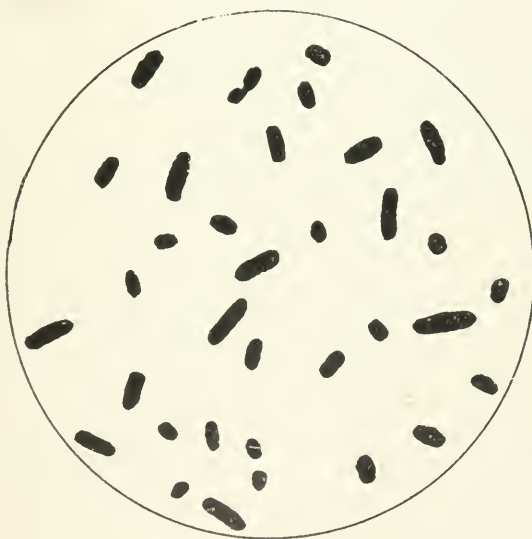
Fig. 3.—Bact. melitensis.

The drawings in Figures 1, 2, and 3 were all made with the aid of a camera lucida from smears of the condensation water from agar slopes after 24-hours' incubation. The smears were stained according to the Gram method without decolorization. × 4,800.

BACTERIUM MELITENSIS

Six strains of the Malta fever organism were available for this study. Strain yf was a strain marked "Micrococcus melitensis (33)" obtained from the American Museum of Natural History. It had been isolated from the arm vein of a human subject in London in 1896. The remaining 5 strains were obtained from the Pathological Division. Their history so far as it could be traced was as follows: Strain aax, marked "M. melitensis Dr. Annett," was obtained in December, 1907, from England; Strain aay, which was marked "M. melitensis Stock 22," and Strain aba, which was marked "M. melitensis Stock," were both received from the Pasteur Institute at Paris some time prior to 1900; Strain aaz, marked "M. melitensis R. A. M. C.," was obtained from the Royal Army Medical College of London, England, in January, 1908; Strain abb, marked "M. melitensis U. S. N.," was obtained from the United States Naval Medical School in March, 1909. Thus all the 6 strains had been cultivated for several years—Strain yf had been cultivated for 21 years—before this study was made. Unfortunately the histories of 5 of the strains gave no information as to whether they were isolated from human subjects or from infected animals. But since some of them were isolated in England, some in France, and one in the United States, and the dates of isolation were distributed throughout a period of a number of years, they may be considered as good representatives of the species. The 6 strains responded alike to all the cultural, biochemical, and serologic tests.

Morphology.—The Malta fever organism like Bact. abortus and Bact. bronchisepticus, is a short, slender rod with rounded ends varying in form according to the conditions under which it was grown. The camera lucida drawings shown in Figure 3 demonstrate that it is unquestionably a rod form, with morphology identical with Bact. abortus. Hence, it should bear the generic name Bacterium. It has a width of about 0.5 mikrons. The longest rod in the drawing is about 1.8 mikrons in length. Bact. melitensis is nonmotile and does not form spores.

Staining.—The organism is easily stained with the ordinary dyes and is decolorized by Gram's method of staining.

Cultural Characteristics.—Bruce[3] states that the first generation on agar slopes required 68 hours at 37 C. to make its appearance. The growth of the old strains on agar slopes, in agar plates, and in agar shake cultures could not be distinguished from the growth of Bact. abortus until after about 7 days' incubation, when the cultures of Bact. melitensis were noticeably browner. The bacterial mass itself was darker and the agar began to take on a brownish tinge which intensified with continued cultivation. Crystals appeared in the agar as in the case of Bact. abortus and Bact. bronchisepticus. The growth on potato also could not be distinguished from Bact. abortus until after about a week's incubation, when it became browner and the potato itself became browner.

Cultures of Bact. melitensis in broth and in whole milk could not be distinguished from cultures of Bact. abortus.

Biochemical Reactions.—Bact. melitensis responded to the biochemical tests exactly in the same manner as Bact. abortus. The identity of the reactions may be compared in Table 1. Most striking is the identity of the reaction to the one quantitative test—the change of hydrogen-ion concentration in broth cultures. Both the Bact. abortus and the Bact. melitensis gave a reduction of the hydrogen-ion concentration equal to about 0.7 or 0.8 P_H.

Agglutination Reactions.—The results obtained by comparing Bact. melitensis with Bact. abortus in respect to agglutination reactions were most surprising. The data are presented in Table 2. The Bact. abortus antiserum was the same as was used for the agglutination of Bact. bronchisepticus. Four of the 6 strains

of Bact. abortus included in Table 2 were partially agglutinated in a dilution of 1 : 1,280 with complete agglutination in lower dilutions. The other 2 strains of Bact. abortus were partially agglutinated in the 1 : 2,560 dilution. Four of the 6 strains of Bact. melitensis were partially agglutinated in the 1 : 1,280 dilution. The other 2 strains were partially agglutinated in the next higher dilution of 1 : 2,560, with a complete agglutination in the 1 : 1,280 dilution. Therefore, Bact. abortus and Bact. melitensis were agglutinated by the Bact. abortus antiserum in exactly the same dilutions.

Thus the comparative study showed Bact. abortus and Bact. melitensis to be identical in morphology, in biochemical reactions, and in their reactions in Bact. abortus antiserum. The only distinction found

TABLE 2

AGGLUTINATION REACTIONS IN BACT. ABORTUS ANTISERUM

Species	Name of Strain	Bact. Abortus Antiserum									Normal Cow Serum	
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:20	1:40
Bact. bronchisepticus..	vl	+	+	0	0	0					0	0
Bact. bronchisepticus..	xb	+	+	0	0	0					0	0
Bact. bronchisepticus..	vp	+	+	+	0	0					0	0
Bact. bronchisepticus..	wn	+	+	+	0	0					0	0
Bact. bronchisepticus..	ww	+	+	+	0	0					0	0
Bact. bronchisepticus..	yc	0	0	0	0	0					0	0
Bact. abortus..........	tq		C*	C	C	C	C	+	0	0	0	0
Bact. abortus..........	wh		C	C	C	C	C	C	+	0	0	0
Bact. abortus..........	wk		C	C	C	C	C	C	+	0	0	0
Bact. abortus..........	wl		C	C	C	C	C	+	0	0	0	0
Bact. abortus..........	aap		C	C	C	C	C	+	0	0	0	0
Bact. abortus..........	wt		O	C	C	C	C	+	0	0	0	0
Bact. melitensis........	yf		C	C	C	C	C	C	+	0	0	0
Bact. melitensis........	aax		C	C	C	C	C	+	0	0	0	0
Bact. melitensis........	aay		C	C	C	C	C	+	0	0	0	0
Bact. melitensis........	aaz		C	C	C	C	C	+	0	0	0	0
Bact. melitensis........	aba		C	C	C	C	C	+	0	0	0	0
Bact. melitensis........	abb		C	C	C	C	C	C	+	0	0	0

* Complete clumping.

in their cultural characteristics was the more intense brown pigmentation of the Bact. melitensis. The next point to be determined was the comparative pathogenic action of the two organisms on guinea-pigs. Four pregnant guinea-pigs were inoculated with a strain of Bact. abortus freshly isolated from an aborted fetus. The inoculated bacteria were from the first growth on agar slope. At the same time 4 pregnant guinea-pigs were inoculated with Bact. melitensis. The strain was yf, which had been isolated from the arm vein of a human subject in London 21 years ago. Within a few days 3 of the animals of each group aborted. Five days after the inoculations were made, one animal from each group was killed and agar slope cultures were made from the organs. In 3 or 4 days the characteristic "dew drop"

colonies made their appearance on both sets of slopes inoculated from the liver, spleen and uterus. No distinction could be found between the growth of the two organisms until the slopes had been incubated for several weeks, when the melitensis slopes showed a more intense brown coloring.

Thirteen days after the inoculations were made one guinea-pig from each group was killed and the serum was obtained for agglutination tests. The results are given in Table 3. The results obtained with the Bact. abortus antiserum from the guinea-pig were the same as with the cow's antiserum. Three of the four tested strains of each species were agglutinated in the 1 : 2,560 dilution, and the other strain of each species was agglutinated in the next lower dilution of 1 : 1,280. The reaction of the Bact. melitensis antiserum was less uniform. The

TABLE 3

AGGLUTINATION REACTIONS OF BACT. ABORTUS AND BACT. MELITENSIS IN HOMOLOGOUS AND HETEROLOGOUS SERUMS

Suspension	Strain	Bact. Abortus Antiserum							
		1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
Bact. abortus........	tq	C	C	C	C	C	C	+	0
Bact. abortus........	wh	C	C	C	C	C	+	0	0
Bact. abortus........	wk	C	C	C	C	C	C	++	0
Bact. abortus........	aap	C	C	C	O	C	++	+	0
Bact. melitensis......	yf	C	C	C	C	C	O	++	0
Bact. melitensis......	aay	C	C	O	C	C	++	+	0
Bact. melitensis......	aaz	C	C	C	C	C	C	++	
Bact. melitensis......	abb	C	C	C	C	++	+	0	0

Bact. abortus suspensions were agglutinated in the 1 : 320 or 1 : 640 dilutions, whereas the Bact. melitensis suspensions were agglutinated in considerably higher dilutions. Two strains were agglutinated in the 1 : 1,280 dilution; one strain in the 1 : 2,560 dilution; and yf, which was the homologous strain, was agglutinated in the highest dilution of 1 : 5,120.

Absorption Tests. — The agglutination tests showed that Bact. abortus antiserum reacts in the same manner on suspensions of Bact. abortus and Bact. melitensis, but that Bact. melitensis antiserum reacts in a different manner on the 2 suspensions. The absorption test was, therefore, applied to the 2 antiserums and their antigens, to demonstrate more clearly the relationship between the 2 species of organisms. The results obtained with the 2 antiserums are given in Table 4. The A series shows the original agglutination reaction of the antiserums,

and the B series shows the reaction of the same antiserums after having been absorbed by the suspensions of the A series. The reactions with abortus antiserum show only one slight distinction between the 2 organisms. It is remarkable that Series Ib and IVb reacted alike to abortus antiserum. It is also remarkable that Series IIb and IIIb reacted alike to this antiserum, but that agglutination took place in higher dilutions than in the Ib and IVb series, showing that the absorbed serum reacts in somewhat higher dilutions on the alternate suspension, whichever suspension may have been first absorbed in Series A. But this is an exceedingly fine point of distinction which would be useless in diagnostic work. The conclusion must be drawn that for practical purposes Bact. abortus antiserum cannot differeniate Bact. abortus from Bact. melitensis.

TABLE 3—*Continued*

AGGLUTINATION REACTIONS OF BACT. ABORTUS AND BACT. MELITENSIS IN HOMOLOGOUS AND HETEROLOGOUS SERUMS

Bact. Melitensis Antiserum							
1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
C	C	C	C	++	0	0	0
C	C	C	+	0	0	0	0
C	C	C	++	+	0	0	0
C	C	C	+	0	0	0	
C	C	C	C	C	C	C	+
C	C	C	C	++	+	0	0
C	C	C	C	C	++	+	0
C	C	C	C	++	+	0	0

But the agglutination reactions with the Bact. melitensis antiserum indicate that the abortus and melitensis antigens are not identical. Series IVa and b show that the melitensis antiserum acts on the melitensis antigen in the same manner that abortus antiserum acts on the suspensions of both species. Series Ia and b show that melitensis antiserum acts on abortus suspensions in characteristically lower dilutions. Series IIIa and b show that even in low dilutions of the melitensis antiserum the melitensis antigen absorbs all agglutinins which are active toward abortus suspensions. Series IIa and b show that after having been saturated with Bact. abortus the melitensis antiserum reacts in the same manner on the melitensis antigen as it would react without having been thus saturated.

The results of the absorption tests can be explained by assuming that both the abortus and the melitensis antiserums contain more than

one agglutinin, that the agglutinins in the two antiserums are alike in kind, but differ in proportion; and that the corresponding agglutinable substances are present in the bodies of the two species of bacteria in different proportions.

PATHOGENIC ACTION OF BACT. ABORTUS, BACT. BRONCHISEPTICUS, AND
BACT. MELITENSIS

A study of the literature shows that there is a similarity between the three organisms under consideration in their choice of location in the animal body.

Bact. abortus takes its name from the most pronounced symptom—the abortion of the fetus, which it produces in cattle. It is known

TABLE 4

ABSORPTION TESTS WITH BACT. ABORTUS AND BACT. MELITENSIS ANTISERUMS

No.	Series	Suspension	Bact. Abortus Antiserum						
			1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
I	A	Bact. abortus........	C	C	C	C	C	+	0
	B	Bact. abortus........	C	C	++	0	0	0	
II	A	Bact. abortus........	C	C	C	C	C	+	0
	B	Bact. melitensis......	C	C	C	C	++	0	
III	A	Bact. melitensis......	C	C	C	C	C	+	0
	B	Bact. abortus........	C	C	C	++	+	0	
IV	A	Bact. melitensis......	C	C	C	C	C	++	0
	B	Bact. melitensis......	C	C	+	0	0	0	

to lead a passive existence in the udders of normal cows which eliminate the organism in their milk. Other organs are affected in other species of animals. Fabyan[8] has shown that in 58 guinea-pigs inoculated with Bact. abortus the spleen was enlarged in 98% of cases; the lymph nodes were enlarged in 95%; the liver was diseased in 75% and lesions were noted in the lungs in 65% of cases. Other organs also were infected in less than 40% of cases, but no lesions of the muscles, heart and digestive tract were found.

Mohler and Eichhorn[9] state: "The most important symptom which is observed among goats affected with Malta fever is the frequency of abortions which result in the course of the disease." Bact. melitensis, like Bact. abortus may lead a passive existence in the bodies of healthy animals. The British Commission[4] found that it was

[8] Jour. Med. Research, 1912, 26, p. 441.
[9] Twenty-eighth Annual Report of the Bureau of Animal Industry, U. S. Dept. of Agriculture, 1911, p. 119.

eliminated in the milk of healthy goats. This Commission made post-mortem examinations of 13 fatal human cases. They isolated the organism from the spleen, liver, and lymphatic glands of 100% of cases in which these organs were examined, and from the kidney of 85% of the cases in which this organ was examined. Mohler and Hart[10] state that in several cases of infected goats the spleen was enlarged, the liver engorged, the kidneys inflamed, and the lymph glands swollen. They also report that in 3 reacting goats the lungs were found to be congested along the borders, and occasionally pneumonic consolidation was present. The British Commission noted that infected goats may suffer from a short hacking cough, indicating an affection of the respiratory tract.

TABLE 4—*Continued*

ABSORPTION TESTS WITH BACT. ABORTUS AND BACT. MELITENSIS ANTISERUMS

Bact. Melitensis Antiserum						
1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
C	C	+	+	0	0	0
+	+	0	0	0		
C	C	+	+	0	0	0
C	C	C	C	C	++	0
C	C	C	C	C	C	+
0	0	0	0	0	0	0
C	C	C	C	C	C	+
C	C	++	+	0	0	0

Bact. bronchisepticus causes primarily diseases of the respiratory tract. It is easily cultivated from lung lesions of diseased guinea-pigs in which it is present in very great numbers. It may also be cultivated from the trachea of healthy guinea-pigs. Smith[1] found the organism occasionally in the uterine horns of guinea-pigs, and then chiefly in association with dead embryos. In our own investigation Bact. bronchisepticus has been isolated from the spleen, liver, heart blood, kidneys and uterus of infected guinea-pigs, as well as from the lungs and trachea.

Considered together, the 3 organisms under discussion may be said to have a predilection for glandular tissue, the respiratory tract and the uterus, with the primary focus of the disease differing with the species of animal infected with the infecting organism. All 3 organisms may lead a passive existence in infected animals.

[10] Twenty-fifth Annual Report of the Bureau of Animal Industry, U. S. Dept. of Agriculture, 1908, p. 279.

DISCUSSION

It is only with great difficulty that Bact. melitensis can be distinguished from Bact. abortus. They are alike morphologically, and no difference could be found in their biochemical reactions. The 2 organisms produced the same results when inoculated into pregnant guinea-pigs. The only distinction between the 2 organisms in cultural characteristics was a more intense brown pigmentation by Bact. melitensis — an insignificant characteristic, which does not appear until after the cultures have been incubated for a week or more. This distinction can be made only when cultures of the 2 species which have been incubated for the same length of time can be compared. The agglutination reactions in Bact. abortus antiserum do not distinguish the two organisms; and the agglutination reactions in Bact. melitensis antiserum can distinguish Bact. abortus from Bact. melitensis only when the agglutinating strength of the serum for both species is known.

The fact that Bact. abortus and Bact. melitensis are serologically so closely related explains Kennedy's[11] discovery that the milk and the blood serum of a considerable percentage of cows in London contained agglutinins for the Malta fever organism in high dilution. This author was unable to explain his findings, but suggested that agglutination of the Malta fever organism by cows' milk was not necessarily specific, or else that the reaction was indicative of infection with the organism in question — the later alternative being an explanation too alarming to be acceptable, although he states that he has heard of two cases of undulant fever in people who have never been out of England, and he thinks it possible that there are other cases undiagnosed.

The very close relationship between Bact. abortus and an organism pathogenic to human beings adds a new interest to the question of the possible pathogenicity of Bact. abortus to human subjects. Considering the close relationship between the two organisms, and the reported frequency of virulent strains of Bact. abortus in cows' milk, it would seem remarkable that we do not have a disease resembling Malta fever prevalent in this country. A possible explanation can be offered. The data presented in the third paper of this series indicates that although there may be numerous abortus-like bacteria in the milk of cows which have aborted, the actual number of virulent bacteria which persist in the milk is not great, or in all probability it is negligible in many cases in which the milk and blood serum contain agglutinins. But the work

[11] Jour. of the Royal Army Med. Corps, 1914, 22, p. 9.

of the British Commission indicates that Bact. melitensis is very abundant in the milk of infected goats, for those investigators were able by cultural methods to demonstrate the organism in the milk of 10% of the goats of Malta. Since infection is dependent on the amount of infectious material, it may be that this difference in the number of bacteria in the milk of the two species of animals may account for our freedom from disease when cow's milk containing Bact. abortus is consumed. On the other hand, are we sure that cases of glandular disease, or cases of abortion, or possibly diseases of the respiratory tract, may not sometimes occur among human subjects in this country as a result of drinking raw cows' milk? It is certain that the agglutination tests, which have been relied on for the diagnosis of Malta fever, have not proved per se whether the infections were due to Bact. melitensis or Bact. abortus.

SUMMARY

Bact. abortus and Bact. bronchisepticus are related. They resemble each other morphologically, culturaly, biochemically, and serologically. Bact. bronchisepticus can be easily distinguished from Bact. abortus, however, by its motility, by its more rapid and abundant growth in all artificial media, by its more intense alkaline reactions, and by its agglutination in Bact. abortus immune serum only in low dilutions.

The organism which causes Malta fever is unquestionably a rod form and should be called Bact. melitensis.

Bact. melitensis is very closely related to Bact. abortus. The only test which has been found to distinguish these two organisms is the agglutination of Bact. melitensis suspensions in higher dilutions of melitensis serum than will agglutinate suspensions of Bact. abortus.

The agglutination tests as they have been used to diagnose infections of Bact. melitensis in goats and human subjects cannot be relied on to distinguish one infection from the other.

HYPERSENSITIVENESS AND ASTHMA, ESPECIALLY IN RELATION TO EMANATIONS FROM HORSES

Arent de Besche

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Some years ago I described[1] a case of marked sensitiveness to horse serum, the chief features of which may be restated here:

1. It concerns a physician, aged 30, who as far back as he can recall would begin to suffer from symptoms of hay-fever and asthma soon after coming in close contact with horses as in driving, going into a stable or circus. He could drive a comparatively short distance only, without symptoms appearing; he could smell whether the horse was nicely groomed or not; in streets where there was much dust mixed with horse droppings symptoms would come on quickly; sitting beside persons whose clothes smelt of horses might bring on asthma.

One aunt and a distant relative have asthma, and one uncle and a cousin have hay-fever, but none of his brothers, sisters or other relatives are affected as he is by horses.

In December, 1908, he was given 2 c c (1,000 units) of diphtheria antitoxin as he had been exposed to diphtheria, and within 5-10 minutes the eyes commenced to itch intensely, the nose to run, and coughing with dyspnea set in. The dyspnea grew worse rapidly, cyanosis developed, the pulse could hardly be counted, and he felt extremely weak. After about 2 hours the symptoms began to subside, and the next day he felt quite well again. A large area of redness with some edema developed about the point of injection.

After this acute intoxication, which was not unlike the anaphylactic shock of the sensitized guinea-pig, the patient could be near horses without any bad effects, but after about 4 months the old sensitiveness returned. About 5 c c of the serum of the patient was injected into the abdominal cavity of a guinea-pig without any ill effects; 48 hours later the animal received an injection of horse serum whereupon anaphylactic shock supervened. This experiment was repeated later with the same result, the guinea-pig now dying from the shock. Somewhat later an attack of asthma was traced to eating sausages containing horse meat as shown by the precipitin test. It was also found that putting a small amount of horse serum on a small scratch in the skin of the arm would be followed in from 2-10 minutes by a local swelling from 1.5-2 cm. in diameter due to edema; further that if a horse was stroked with a finger which was then applied to the conjunctiva an intense redness would appear in a few minutes. Dropping horse serum in the eye would also cause a brief redness. Exposure to other animals (cattle, sheep, dog, cat) was without effect; the odor of rabbits and guinea-pigs might provoke mild symptoms, and rabbit serum caused a reaction in the skin.

Similar observations have been made by others, notably Gillette,[2] and I have had the chance to observe several cases of "horse asthma," which I shall report in brief.

2. A woman, aged 36, had occasional attacks of asthma in childhood and when she became 10 years old it was noticed that they would come on when driving, going into stables and in streets where there was much traffic; when still older, asthma would come on in the winter also, and since reaching her 30th year the attacks have been worse. She has no hay-fever, but a sister has, and an uncle had asthma in his old age. By staying 30 minutes in a stable with horses, itching and running of the eyes would set in, also running from the nose, cough and typical asthma. Horse serum gave a well marked skin reaction and she also developed conjunctival redness on touching the conjunctiva with a finger with which a horse had been touched.

3. A woman, aged 24, always acquired redness of the eyes, nasal irritation, and asthma when driving with horses for some time. No hay-fever, but a sister has, and an aunt has bronchial asthma. After remaining in a stable for 10 minutes the nose began to flow, the conjunctivae became irritated, the lips blue, and there was some coughing with râles over the lungs; complete recovery in 3 hours. Horse serum gave a marked skin reaction (edema), which disappeared in 1½ hours; touching the eye with a finger which had just touched a horse caused marked redness, increased flow of tears, and sneezing. The only other animals, contact with which would produce any of these symptoms, were the rabbit and the guinea-pig.

4. A man, aged 45, often had asthma since childhood, and since his 7th year the attacks would come on when going into stables and on driving; attacks would also come on without coming into close contact with horses, but only in the city, never in the country. Several members of his family were asthmatics or had hay-fever. Since his 14th year he has been a sea-faring man, and never has any attacks while at sea. If attacks develop when ashore, they disappear promptly on going out on the sea. After staying 20 minutes in a stable conjunctival and nasal irritation developed with difficult breathing and râles over the lungs. No skin reaction with horse serum but redness and flow of tears came on soon after touching the eye with a finger that had touched a horse shortly before. In this case anaphylactic shock developed in a guinea-pig injected with horse serum 48 hours after having been injected with 5 c c of the patient's serum.

5. A woman, aged 25, commenced to suffer from asthma when 16 years old, without any warning, and without ever before having felt any peculiar symptoms when in contact with horses. The attacks grew worse, and 4 years later when in a ridng school a very severe asthmatic state developed and blisters came out all over the face. This repeated itself after new attempts to take riding lessons. She now was so sensitive that by merely standing next to a person whose clothes had the least bit of horse smell, the eyes would begin to itch and pain and asthma would come on. Once an attack came on after sleeping in a bed covered with an overcoat used in driving. If on returning from driving her husband kisses her, blisters develop in the face and itching of the eyes. Her attacks are brief. She has no hay-fever and there is no history of asthma or hay-fever in the

2 Jour. Am. Med. Assn., 1905, 52, p. 580.

family. She gives well marked skin reaction to horse serum and the eye reacts promptly when touched with a finger that has come just barely in contact with a horse.

6. A man, aged 42, had asthma since his 10th year, but it was not until his 18th year that he understood that the asthma was connected in some way with horses. A stay of 5 minutes at the side of a horse was sufficient to bring on an attack, but attacks come on which cannot be traced to horses, especially in cold damp weather. The attacks are associated with pain in the abdomen and vomiting. No hay-fever; no asthma or hay-fever in the family. Both lungs are emphysematous, the chest barrel shaped. Horse serum does not cause any skin reaction, but eye reaction to the finger test is prompt.

7. A man, aged 22, has had asthma since his 10th year, dampness, dust, and cold weather apparently bringing on the attacks. When 18 years old, however, he noticed that close association with horses, cattle, and wet dogs would cause attacks. He has hay-fever, and his father has bronchial asthma. Horse serum does not cause any skin reaction, but touching the eye with a finger which has just been in contact with a horse is followed in 5 minutes by itching, redness, tears, but no sneezing.

8. A man, aged 35, would get itching of the eyes, lacrimation, sneezing and coughing with dyspnea whenever in contact with horses. This has been the case since his 5th year. Eating strawberries, either fresh or as jam, would be followed by pressure in the chest and dyspnea. He is a sea captain, and never has asthma when on the sea. The skin reaction with horse serum could not be obtained, but the eye reacted promptly to the finger test.

In addition to these cases, in which the asthma was known to be precipitated by contact with horses, I have studied also a number of asthmatic patients, persons who could not trace their attacks to association with horses or other animals.

9. A woman, aged 34, has had asthma as long as she can remember; as she did not associate her attacks with anything in particular, I asked her to stay in a stable for awhile. After 10 minutes she came out with red, overflowing eyes, nasal discharge, and asthmatic breathing—recovery in 5-6 hours. The eye and skin tests gave only mild reactions. Subsequently I learned that she was very much better so long as she kept away altogether from horses.

10. A man, aged 23, with asthma since childhood, stated that the attacks often came on some 2-3 hours after meals especially after supper. His diet was changed so that he had no bread for breakfast and supper, only mush and milk. On this diet for 3 months he had no attacks, but soon after commencing to eat bread again the asthma reappeared; on restricting him to hard bread only the asthma grew better again. Contact with horses had no influence. No eye or skin reactions.

11. A man, aged 27, began to have asthma when 23 years old; the attacks usually came on at night and would continue for about 2 weeks. He would be well for long periods, no reason being apparent for the asthma which was not in any way connected with horses. The mattress and pillow on which he slept were filled with horse, cow, and cat hair besides wool and were not very clean; on changing to mattress and pillow filled with vegetable matter great improvement took place. He gave an eye reaction by touching the eye with a finger

which had stroked a cat. Holding a cat for half an hour caused the patient to feel badly and to have mild asthma. He does not have hay-fever, but members of his family have hay-fever and asthma.

I have examined 18 cases of ordinary bronchial asthma without being able to discover any relation to horses or to find any exciting cause.

Of the 9 cases described in which the asthma seemed to be dependent in some way on horses, four (Cases 1, 2, 3, and 5) gave a positive skin reaction to horse serum. By scratching the skin and then placing a little horse serum on the scratch, a red spot would appear in from 2 to 10 minutes and persist for an hour or so. All the cases gave eye reaction, that is, touching the conjunctiva with a finger which had just touched a horse would cause a redness to appear in a few minutes with itching, sometimes edema and sneezing, with increased secretion.

As it naturally would be advisable to apply some test in the case of persons suspected of being sensitive to horse emanations, before injecting horse serum, I have made the eye and skin reactions with horse serum on 30 students, all negative; on 12 hay-fever patients, all negative both in and out of the hay-fever season; and as mentioned, on 18 patients with bronchial asthma, also all negative. This result indicates that a positive skin or eye test with horse serum points very definitely to the existence of sensitiveness to horse proteins.

The question now arises, Is it safe to inject asthmatics who give negative skin and eye reactions with horse serum? Gillette and others warn against injecting all asthmatics. I know of 6 hay-fever patients who have been injected with horse serum without any ill effects, and also of 3 asthmatics. Hence it would seem to be quite safe to inject hay-fever and asthma patients who are not sensitive to horse emanations, but in the case of such as are sensitive the injection is unquestionably very dangerous. For this reason all asthmatics and hay-fever sufferers should be tested with the eye reaction and the skin reaction before horse serum is injected, as they may not be aware that their condition stands in any relation to horses.

The question arises whether persons previously injected with horse serum would give any skin or eye reaction. It has been found by Dr. Schönfelder that of 15 such persons not a single one gave a reaction. Of course this does not mean that reinjection of horse serum in such a person never will give rise to any severe symptoms; on the other hand, it is probably the case that in a person once injected with horse serum who gives a skin or eye reaction, a reinjection very likely would cause

severe symptoms. In this connection a case described by Walker[3] is of great interest: A woman, aged 20, who had not had asthma before, received an injection of horse serum without ill effect; 2 weeks later she began to have attacks of asthma whenever she came into close contact with horses; alcoholic extract of horse dandruff gave a skin reaction. This case suggests that if a large number of skin and eye tests were made soon after injection of horse serum a few reactors would undoubtedly be found.

I have had occasion also to make some observations on somewhat different forms of sensitiveness.

12. A man, aged 30, was always unable to play with cats without irritation of the eyes; occasionally blisters of the skin would develop also. He has no asthma and there is no asthma or hay-fever in the family. The mere presence of a cat would not affect him, but if a finger which had just touched a cat was placed on the conjunctiva tears would begin to flow and there would be itching
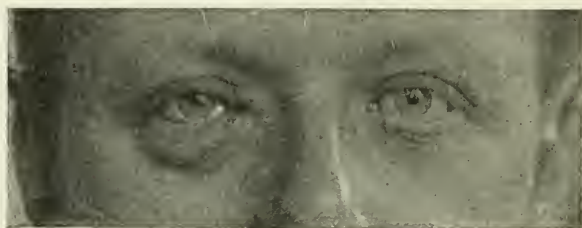
Fig. 1.—Lacrimation, itching and redness of the eyes with gradual chemosis and edema of the tissues; the effect of contact of the finger which had just touched a cat on the conjunctiva.

and redness of the eye, and gradually chemosis and edema of the tissues at the inner angle would develop (Fig. 1). These changes would disappear in about 3 hours. If the hair of a cat gets into the eye the same changes occur, and if a cat scratches the skin a large blister forms. Five c c of blood were injected into a guinea-pig; 48 hours later cat serum was injected and there resulted an immediate anaphylactic shock with death. No further experiments were permitted by the patient.

13. A man, aged 36, has had hay-fever in mild degree since puberty; there are 2 cases of hay-fever in the family. He is not sensitive to horses and gives no reaction, either eye or skin; he once received an injection of diphtheria serum without any ill effects. For many years he has had diarrhea with colic in the summer-time, without any known cause except that these disturbances were closely connected with the attacks of hay-fever. If he went into the mountains in the summer both the hay-fever and the diarrhea would disappear.

14. A man, aged 32, noticed since early childhood that when among flowers he would sneeze and itch in the eyes; the smell of flowers especially of roses had the same effect. Whenever exposed to such odors for 15-20 minutes, colic

[3] Jour. Med. Research, 1917, 36, p. 427.

and diarrhea come on, sometimes to his great embarrassment. Whenever he goes to a party he remains as far away from the floral decorations as possible. While under my observation, he kept his nose for 15 minutes in a bunch of roses with the result that diarrhea came on. Scratching the skin and then putting some rose juice on the scratch resulted in an insignificant swelling.

Some of the patients whose asthma was connected with horses seemed to be sensitive only to horses, while others were influenced also by climate and other conditions. In some cases the patients themselves were not aware of their sensitiveness to horses, but ascribed their illness to dampness, etc. Some of them also suffered from hay-fever (Cases 7 and 8); in a few cases other animals seemed to influence the asthma. In some instances the patients could recall that horses made them feel badly ever since their earliest days; others had noticed it since their 10th year or so; others again since their 16th or 18th year.

It is quite impossible to determine whether this sensitiveness is acquired or inborn. It would seem likely that in some cases at least the condition is inborn, but we know that it may be acquired as shown by the woman whose asthma developed after the injection of horse serum (Walker). It is possible that eating horse meat for a long time also may have an influence. The occurrence of "horse asthma" in more than one member of the same family has not been noticed, but families in which bronchial asthma and hay-fever occur seem to be especially predisposed. It is noteworthy that sometimes there are intestinal disturbances associated with the asthma. Attacks of vomiting and pain in the abdomen may occur at the same time as the asthmatic attacks or independently. In Walker's 40 cases of asthma, 3 or 4 presented such symptoms. It is of interest to note here that vomiting may occur in the typical anaphylactic shock. Diarrhea may also develop in connection with the asthma; in one patient diarrhea and hay-fever were associated, coming on and disappearing together. Here may be mentioned again the case of sensitiveness to roses, in which sudden attacks of diarrhea would come on. In anaphylactic shock there often is congestion, hemorrhages, and excessive formation of mucus (enteritis anaphylactia), and Schultz[4] observed that a piece of intestine in Ringer's fluid would contract if horse serum was added to the fluid and that the contraction was far stronger if the intestine came from a sensitized animal, indicating that the antigen uniting with the antibodies in the tissues greatly increase the muscular contractions.

[4] Jour. Pharmacol. and Exper. Therapeutics, 1910, 1, p. 549.

What sort of substances are the patients in question sensitive to? Most likely some form of protein as illustrated by the sensitiveness to pollen in hay-fever. We know that in some cases as in my Case 1 and the cases described by Willey, Gillette, Bacon and Wright, typical shock may follow injection of horse serum in persons sensitive to horses and that there may supervene a state of antianaphylaxis which may last for 3 or 4 months; furthermore that, as in Cases 1, 4, and 12, sensitiveness to horse serum may be transferred to guinea-pigs with the serum of the patients, which constitutes a perfect analogy to passive anaphylaxis. All these facts point to a state of sensitiveness to foreign protein, which may be contained in the sweat and dandruff of the animals concerned. That such must be the case is indicated by the skin and eye reactions which I have described. But it is hard to explain how the patients are influenced when attacks develop without being in close contact with the animals. It seems as if a diffusible substance was in the air. Another difficult question is, How do the antibodies, which are regarded as essential for the reactions of hypersensitiveness develop in these individuals? Here we are reduced to conjecture and it lies near at hand to assume the existence of some anomaly of protein assimilation. Be that as it may, the conception that these forms of asthma are expressions of hypersensitiveness gives us a basis for rational treatment, namely, by exclusion of the substances concerned, the antigens, on the one hand, or by a gradual immunization with the antigens on the other hand, in order to bind the antibodies without causing noticeable symptoms, as adopted recently with promising results by Walker.[3]

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